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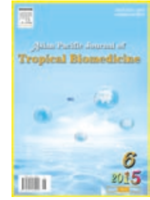

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The effect of renal stones on serum adenosine aminohydrolase and AMP-aminohydrolase in Malaysia

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ABSTRACT

Objective: To verify possible associations between adenosine aminohydrolase (ADA) and AMP-aminohydrolase (AMPDA) to E3 SUMO-protein ligase NSE2 (NSMCE2) in patients with renal stones. And to isolate, purify and characterize ADA in patients with renal stones and healthy group.
Methods: A total of 60 renal stones patients and 50 control were enrolled in a case-control study. The blood urea, creatinine, uric acid, protein, albumin, ADA and AMPDA were measured by colorimetric tests. The serum NSMCE2 was measured by ELISA.
Results: Serum ADA, AMPDA and specific activity of enzymes showed significant decrease ($P < 0.05$) in patients with renal stones compared to control group, mean levels of sera NSMCE2 and uric acid had a significant increase ($P < 0.01$ and $P < 0.05$, respectively) in patients compared to control group.
Conclusions: The present study suggests that ADA, AMP deaminase and NSMCE2 can be used as a indicator to monitor the DNA damage and inflammation disorders in the patients with kidney stones.

1. Introduction

Simple renal stones are common in adults, particularly in the old population. They are crystal accumulations made in the kidneys where recurrence of stone formation is common[1]. It is a very common kidney disorder all over the world, an assessed lifetime risk of 2%-5% in Asia and 8%-15% in Europe[2]. Thus around 40% of the stone formers do not produce another stone. Stone formers with systemic diseases such as cystinuria, the most important hyperoxaluria, and primary hyperparathyroidism have more risks of recurrence[3]. The stones can form anywhere in the urinary system, from kidneys to the bladder but in the industrialized and affluent societies, they are generally restricted to the kidneys[2]. The information on chemical configuration of urinary lithiasis is a very important step in the treatment of this disease[3].

Adenosine aminohydrolase (ADA) is a polymorphic enzyme involved in purine metabolism and it is necessary in the purine salvage pathway[4]. It catalyzes the irreversible deamination of 2'-deoxyadenosine and adenosine to deoxyinosine and inosine. It is found in a wide variety of prokaryotes and eukaryotes in different forms[5]. It is widely distributed in human tissues and shows highest activity in lymphoid tissues and it is necessary for the proliferation, maturation and function of lymphocytes, specifically for T lymphocytes[5,6]. The ADA is widely distributed in animal and human tissues[4]. It is present in the cytoplasmic fraction and a certain amount is located in the nucleus[7]. The physiological function of ADA is critical in controlling the effects of these metabolites on immunological, neurological and vascular systems[8]. The AMP-aminohydrolase (AMPDA) is a key enzyme of nucleotide breakdown involved in regulation of adenine nucleotide pool in the liver and energetic metabolism in mammalian cells[9].

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Also, it plays a crucial role in the synthesis of guanine nucleotides and in the provision of anaplerotic substrates for the Krebs cycle[9]. The highest ADA activity has been reported in lymphoid and fatty tissues, liver, skeletalmuscle, and heart[10].

Small ubiquitin-like modifier (SUMO) proteins are a family of main proteins belongs to the ubiquitin and ubiquitin-like protein family[11]. SUMOylation is a part of important regulatory mechanisms that adjust proteins in the nucleus and control multiple cellular processes such as nucleo-cytoplasmic signal transduction, apoptosis, stress responses, protein stability, subcellular localization of proteins, protein-protein interactions, protein-DNA interactions, and transcriptional activity of transcription factors and progression through the cell cycle[12-14]. Very few studies on patients with renal disease have been reported, which are controversial with respect to serum ADA and AMPDA activity[15,16]. On the other hand no one has reported on serum ADA and AMPDA activities in patients with renal stones. Thus, in the current study, we aimed to observe the correlation of serum ADA and AMPDA activities to E3 SUMO-protein ligase NSE2 (NSMCE2) patients with renal stones and assess the possibility of a new biomarker for the evaluation of kidney damage. Also the study aimed to give a comprehensive study of adenosine aminohydrolase including separation, characterization and purification from sera of patients with renal stone and control using several biochemical techniques.

2. Materials and methods

The present work had been done under the support from the International Islamic University Malaysia (IIUM), the Research Management Center Grant Scheme Project No. IIUM/504/5/29/1. The IIUM Research Ethics Committee (IREC) operates in according to Declaration of Helsinki International Conference of Harmonization Goof Clinical Practice Guidelines (ICH-GCP), Malaysia Good Clinical Practice Guidelines and Council for International Organization of Medical Sciences (CIOMS) International Ethical Guidelines, No. IIUM/305/14/11/2/IREC 300 on October 2014. A total of sixty patients with renal stone and fifty healthy as control were included in this study. These patients were hospitalized at government health clinics in Kuantan–Pahang. Five milliliter of blood sample was collected and the blood was allowed to clot for at least 10-15 min at room temperature, centrifuged for 10 min at 3 000 r/min. The levels of serum urea, creatinine, uric acid and albumin were measured by spectrophotometric methods supplied by Randox kits. The NSMCE2 assay employs the quantitative sandwich enzyme immunoassay.

2.1. Determination of ADA and AMPDA activities and total protein

The ADA activity was determined according to Giusti method[17]. The activity was measured using spectrophotometer. ADA unit was defined as the amount of enzyme which forms one micromole of ammonia in one minute. Determination of AMPDA activity was carried out according to Gromashevskaia method[18]. Serum total protein was estimated by Lowery *et al*. method[19].

2.2. Partial purification of ADA

2.2.1. Ammonium sulfate precipitation

Protein was precipitated using solid ammonium sulfate[20]. The supernatant was separated by cooling centrifugation at (10 000 r/min) for 30 min. Protein concentration and ADA activity was measured.

2.2.2. Dialysis

The dialysis sac containing the suspension in Step 2.2.1. was dialyzed overnight at 4 °C using sodium bicarbonate. The final volume measured and the protein concentration was assessed, and the ADA activity was determined[17].

2.2.3. Gel filtration chromatography

Gel filtration chromatography was used for partial purification of ADA enzyme, and two milliliter of the sample was used to a column (2 × 87 cm) comprising sephadex G-100 to 85 cm height. Elution was carried out at flow rate of 70 mL/h, using phosphate buffer (pH = 6.5) as eluent, the fractions were collected; ADA activity and the protein concentration were measured. All steps were done at 4 °C.

2.3. Kinetic parameters (Km and V max)

2.3.1. Effect of substrate concentration

ADA enzymatic reaction was carried out in optimum reaction condition using altered concentrations of adenosine as a substrate (0.002, 0.005, 0.010, 0.015, 0.020, 0.025, 0.030, 0.040 and 0.050 mmol/L). The correlation between each substrate concentration and the enzyme activity was plotted in order to determine the optimum substrate concentration for each enzyme activity. Then the values of Km and Vmax for ADA to substrate were determined by using the Lineweaver-Burk plot (the relationship between 1/V versus 1/Substrate).

2.3.2. Effect of the pH

The enzymatic reaction was carried out via buffers with different pH (4.5, 5.5 ,6.0 , 6.5 ,7.5 , 8.5, 9.5 and 10.5) for ADA. The optimum pH was estimated by scheming the relationship between the enzyme activities versus the pH values.

2.3.3. Effect of the temperature

ADA enzymatic reaction was carried out in optimum reaction condition using different temperatures (20, 25, 30, 35, 37, 40, 45, 50, 55 °C). The optimum temperature was evaluated by plotting the correlation between the enzyme activities versus the temperature values.

2.3.4. Thermodynamic parameters

The thermodynamic parameters of the transition state [Ea, the enthalpy of transition state (ΔH^*), the transition state of free energy change (ΔG^*), the change in entropy of the transition state (ΔS^*)] were estimated from Arrhenius plot of ln K_{+1} values against (1 / T) values, which gives a linear correlation according to the Arrhenius equation:

$$\ln K_{+1} = -Ea / RT + A$$

The activation energy of the binding reaction was calculated from the slope of the straight line.

Where, A represents for Arrhenius constant, Ea refresents for the activation energy, R represents for the gas constant and T represent for absolute temperature.

2.3.4.1. Determination of the activation energy and temperature coefficient

The relation between the activation energy and temperature coefficient were determined by using the equation:

$$Ea = 2.3\ RT_2T_1 \log Q10/10$$

Where, Q10 is the temperature coefficient, R is the gas constant, T is the temperature, Ea is the activation energy.

2.3.4.2. Determination of the ΔH^*, ΔS^* and ΔG^*

The enthalpy of transition state (ΔH^*) was determined from the following equation:

$$\Delta H^* = E - RT$$

The transition state of free energy change (ΔG^*) was calculated from the following equation:

$$\Delta G^* = -RT \ln K_{+1} + RT \ln KT / h$$

Where, K is Boltzmann constant (1.38 × 10-23 J/K), h is Plank constant (6.62 × 10-34 J·sec).

The change in entropy of the transition state (ΔS^*) was calculated from the following formula:

$$\Delta S^* = (\Delta H^* - \Delta G^*) / T$$

2.4. Statistcal analysis

All statistical analyses in studies were performed using SPSS version 20.0 for Windows. Descriptive analysis was used to show the mean ± SD of variables. The significance of difference between mean values was assessed by student's *t*-test. The probability $P <$ 0.05 means significant, $P > 0.05$ means non-significant.

3. Results

A total of 60 of patients with renal stone and fifty control were included in the current study. The present study showed that blood urea, serum creatinine, serum albumin and total serum protein have been non significantly different ($P > 0.05$) compared to control group (Table 1). It also indicated a significant increases in serum NSMCE2 and uric acid ($P < 0.01$) and ($P < 0.05$) respectively for diagnosed patients compared to control group (Table 1).

The current study showed that mean ADA and AMPDA activities and specific activity have been significantly decreased ($P < 0.05$) in patients with renal calculi compared to control group (Table 2).

There were a significant different correlations between NSMCE2 with ADA, AMPDA and uric acid in patients with renal stones compared to control group (Table 3).

Tables 4 and 5 showed the purification by ammonium sulfate, making it most appropriate for the preparation of a protein that is vulnerable to proteolytic degradation such as ADA in patients and control groups. The specific activity was a slight increased after dialysis. This may possibly be due to the exclusion of the small molecules and increase the purification of enzyme. Figures 1 and 2 show that there is mainly one peak by gel filtration separations for patients and control group respectively. The specific activity of the enzyme was increased in 11.12 folds than the activity in initial extract (Table 4) for patients group and specific activity of the enzyme was increased in 10.04 folds for control group.

The total activity of ADA (78.75 IU/L) with 59.65% recovery for patients group, while the total activity of ADA (105.00 IU/L) with 58.00% recovery for control group are shown in Tables 4 and 5, respectively.

The activity of the ADA was measured in the presence of different concentrations (0.002, 0.005, 0.010, 0.015, 0.020, 0.025, 0.030, 0.040, 0.050 mmol/L) of adenosine as a substrate. It was found that the maximum activity of the enzyme was obtained by using (0.020 mmol/L) of adenosine in both patients and control groups (Figure 3).

Figure 4 showed Lineweaver-Burk plot by plotting the reciprocal of the initial velocity versus the reciprocal of the substrate concentration. A linear relationship was obtained with a Vmax

Table 1

The age, serum urea, creatinine, protein, albumin, uric acid and NSMCE2 in patients group and control group (mean ± SD).

Group	Age (year)	Blood urea (mg/dL)	Serum creatinine (mg/dL)	Serum protein (g/dL)	Serum albumin (g/dL)	Serum uric acid (mg/dL)	NSMCE2 (pg/mL)
Patients group (*n* = 60)	52.42 ± 10.19	40.25 ± 7.57	1.16 ± 0.42	7.65 ± 0.43	4.39 ± 0.58	6.90 ± 0.94*	80.41 ± 8.45**
Control group (*n* = 50)	50.27 ± 8.21	37.89 ± 8.36	1.10 ± 0.24	7.68 ± 0.32	4.61 ± 0.57	5.32 ± 0.53	70.04 ± 107.81

*: P <0.05, **: P < 0.01 compared to control group.

Table 2

The serum ADA, AMPDA and specific activity in patients and control groups (mean ± SD).

Group	ADA		AMPDA	
	Activities (IU/L)	Specific activity (IU/mg)	Activities (IU/L)	Specific activity (IU/mg)
Patients group (*n* = 60)	11.63 ± 4.00*	0.15 ± 0.05*	9.51 ± 3.45*	0.12 ± 0.06*
Control group (*n* = 50)	17.56 ± 7.48	0.24 ± 0.09	14.22 ± 5.37	0.19 ± 0.08

*: P <0.05 compared to control group.

(15.70 IU/L) and Km value of (0.047 mmol/L) in control group and a Vmax (12.77 IU/L) and Km value of (0.066 mmol/L) for patient with renal stones. To our knowledge no previous study has purified and determined kinetic study of ADA in patients with renal stones.

The pH (4.5, 5.5, 6.0, 6.5, 7.5, 8.5, 9.5, 10.5) effect on ADA activity has been studied in patients and control group (Figure 5A). The activity of partial purified ADA increased according to the incubation temperature until it reached maximum at 37 °C for patients and control groups (Figure 5B). The role of enzyme catalyzed reactions, similar to chemical reaction, increased with temperature.

The thermodynamic parameters of the transition state were estimated from Arrhenius plot of ln K_{+1} values against (1/T) values (Figure 6).

A linear relationship was obtained with the activation energy of 4 854.64 cal/mol and the temperature coefficient of 1.84 in patients group and the activation energy of 5 955.44 cal/mol and the temperature coefficient of 1.98 in control group.

Table 6 shows the values of ΔH^*, ΔS^* and ΔG^* in transition state for enzyme–substrate ([ES]) complex reaction.

Table 3

Correlation between NSMCE2 with several biochemical parameters in patients and control groups.

Characteristics	NSMCE2 in patients (pg/mL)		NSMCE2 in control (pg/mL)	
	Pearson correlation	Sig.	Pearson correlation	Sig.
ADA (IU/L)	-0.76	0.01	0.09	N.S
AMPDA (IU/L)	-0.80	0.01	0.11	N.S
Uric acid (mmol/L)	0.62	0.01	0.10	N.S

Sig.: Significance (2-tailed). N.S: Not significant.

Table 4

Partial purification steps of ADA from patients with renal stones

Purification stage	Volume (mL)	Activity (IU/L)	Total activity (IU)	Total protein (g/L)	SA (IU/ min/mg protein)	Yield (%)	Folds of purification
Crude	10.0	13.20	132.00	75.98	0.17	100.00	1.00
Ammonium sulfate supernatant	4.5	24.35	109.58	49.78	0.49	83.02	2.88
Dialysis	2.5	36.55	91.38	26.23	1.39	69.23	8.18
Sephadex G-100	21.0	3.75	78.75	1.98	1.89	59.65	11.12

SA: Specific activity.

Table 5

Partial purification steps of ADA from control group.

Purification stage	Volume (mL)	Activity (IU/L)	Total activity (IU)	Total protein (g/L)	SA (IU/ min/mg protein)	Yield (%)	Folds of purification
Crude	10.0	18.10	181.00	77.65	0.23	100.00	1.00
Ammonium sulfate supernatant	5.4	28.44	153.58	51.43	0.55	84.85	2.39
Dialysis	3.2	39.22	125.50	27.00	1.45	69.34	6.30
Sephadex G-100	21.0	5.00	105.00	2.16	2.31	58.00	10.04

SA: Specific activity.

Table 6

Thermodynamic parameters at transition state in patients with renal stones and normal subjects

Case	Ea^* (KJ/mol)	ΔH^* (KJ/mol)	ΔG^*(KJ/mol)	ΔS^*(J/mol·K)
Patients group	20.31	17.73	96.33	-253.55
Control group	24.92	22.34	87.46	-210.06

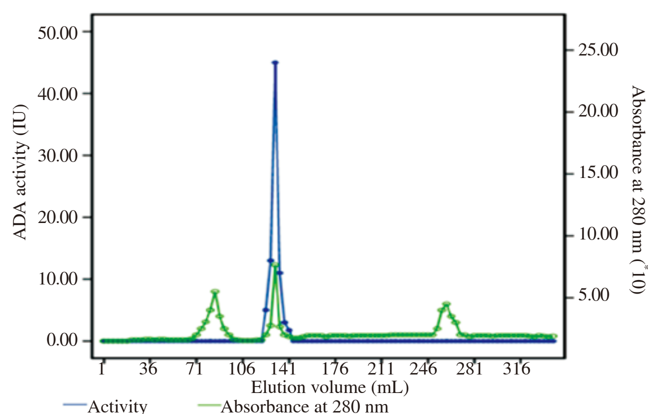

Figure 1. Elution profile of ADA from sera of patients with renal stones using Sephadex G-100, flow rate (70 mL/h) and column (2 × 87 cm).

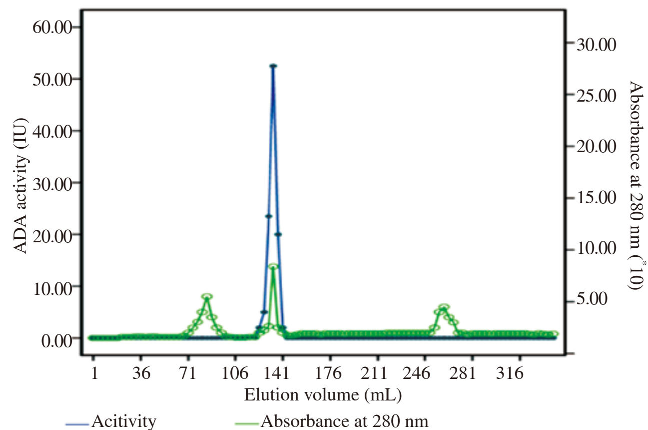

Figure 2. Elution profile of ADA from sera of control group using Sephadex G-100, flow rate (70 mL/h) and column (2 × 87 cm).

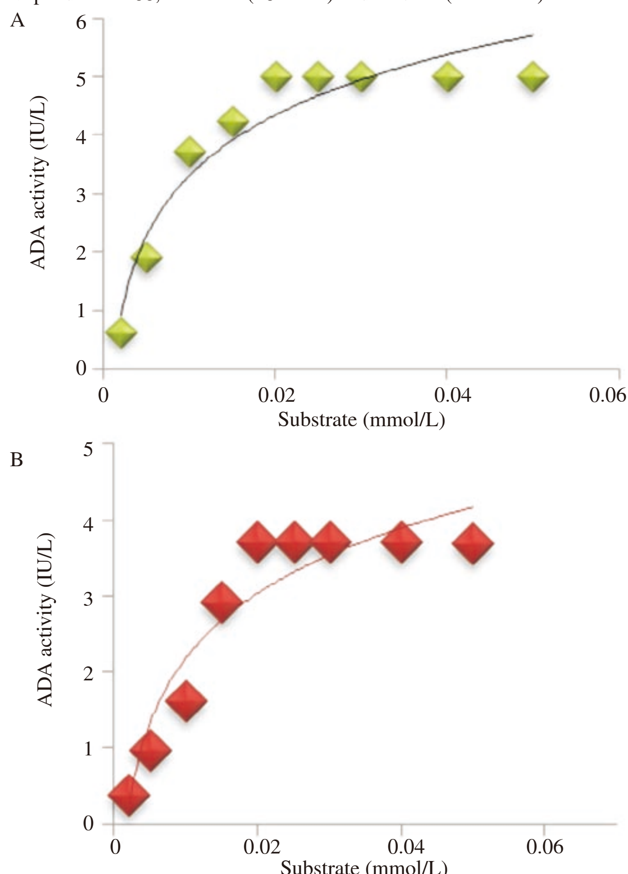

Figure 3. Effect of substrate concentration on ADA activity.

A: Control group; B: Patients group.

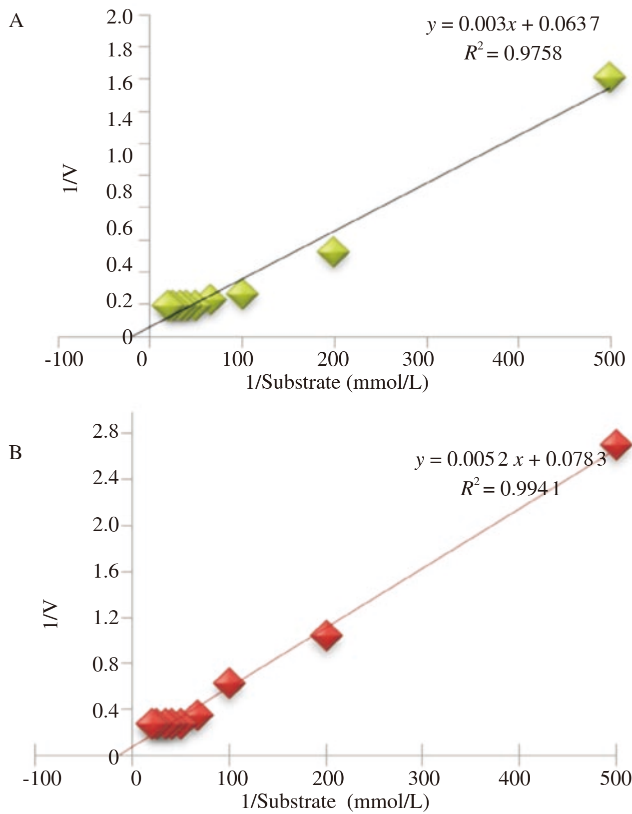

Figure 4 . Determination of Km and Vmax for ADA of in partial purified ADA using Lineweaver-Burk plot.

A: Control group; B: Patients group.

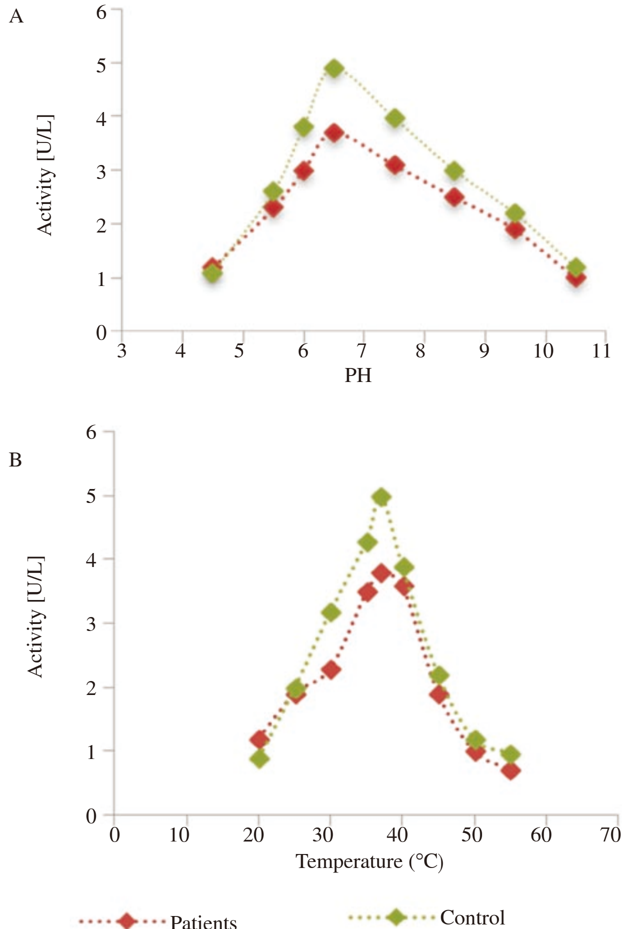

Figure 5. Effect of temperatures and pH on ADA activity.

A: Effect of pH; B: Effect of temperatures.

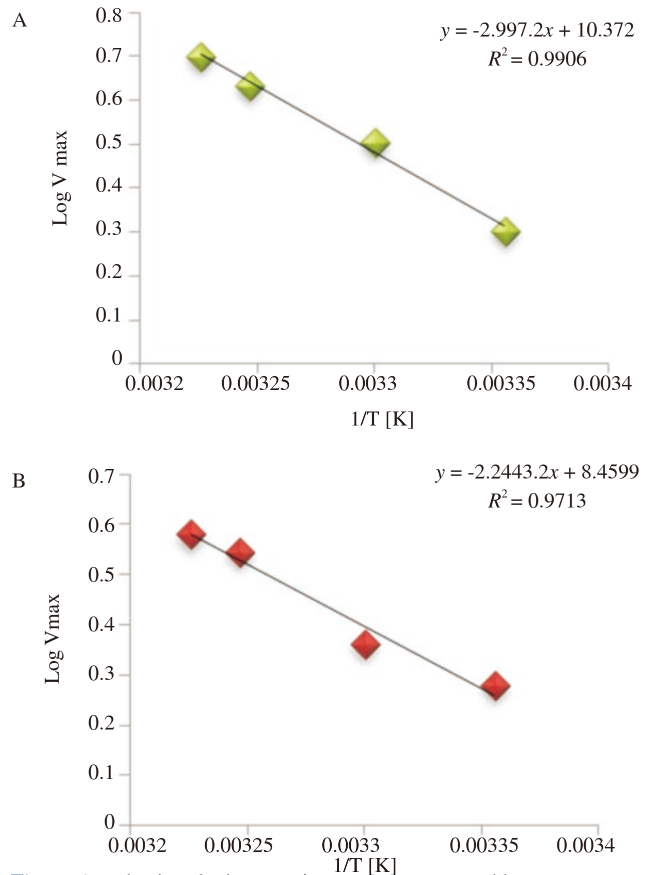

Figure 6. Arrhenius plot between invers temperature and log Vmax.

A: Control group; B: Patients group.

4. Discussion

The present result come to an agreement with other studies which hypothesized that renal stones and urinary calculus have been associated with increased levels of uric acid in the blood[21,22]. Several papers have described elevated uric acid is an independent risk factor for kidney disease in the general population[23,24]. Another clinical study found that lowering uric acid in patients with renal disease and asymptomatic hyperuricemia resulted in less progression of their renal disease[25]. Uric acid is the main product of catabolism of the purine nucleosides. Usually two thirds to three fourths of it is excreted via the kidneys, and residual by the intestines. Renal control of uric acid is complex[26]. Glomerular filtration of practically all uric acid occurs in capillary plasma incoming the glomerulus followed by reabsorption of about 98 to 100% of uric acid in proximal convoluted tubular. The following secretion of half of reabsorbed uric acid takes place in distal portion of proximal tubule and post-secretory reabsorption of 40% of secreted uric acid in distal tubule[27].

Several studies dedicated on ROS-mediated SUMOylation, which is one of the posttranslational differences, which may be

due to possible implications on vascular inflammation[28,29]. SUMOylation regulates fundamental cellular processes such as apoptosis, response to cellular stress and cellular proliferation[30]. The SUMO modification of proteins has been submitted to adjust various physiological progresses, such as stress responses, transcriptional regulation, and protein localization.Recent studies indicate a role for SUMOylation in the regulation of inflammation[28]. 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. Furthermore the rise in NSMCE2 may possibly be due to amplified free radicals effects which subsequently leads to an increase in damage in DNA and because of the dynamic role of this enzyme in the treatment of damage made in the DNA. Certainly adenosine and its metabolites weaken lymphocyte function and proliferation in a dose dependent manner, lower ADA intracellular adenosine concentration and also inactivates T cells[31]. The AMPDA is the rate-limiting step for entry into the purine nucleotide cycle and catalyzes the conversion of adenosine monophosphate to inosine monophosphate[31]. The insufficiency of ADA activity leads to severe immunodeficiency disease in which T-lymphocytes and B-lymphocytes do not develop appropriately, which means that ADA is a non-specific indicator of the activation of the T and B cells, which has a significant role in the etiology of numerous disease[32].

The results showed that maximum enzyme activity was at pH 6.5 in partial purified ADA in patients and control groups. The velocity of enzyme-catalyzed reactions depends on pH. Enzymes have optimum pH and frequently give bell-shaped curves of velocity against pH, even though other shapes have been observed[20]. The decrease in ADA activity at low pH may be due to consequence of pH environment of reaction in ionic groups in active site or changing in ionic state for substrate or complex enzyme-substrate at the concentration of substrate above than Km, if the substrate concentration is small, it will depend on enzyme[33]. The result of the present study showed that, above and below optimum temperature, serum ADA enzyme is more heat stable in patients than in normal. This might be due to the alterations in the ratio of isoenzymes in the serum of patient for the reason that isoenzymes have not the same stability to temperature. The tertiary structure of an enzyme is kept principally by a amounts of non-covalent links. When molecule absorbs much energy the tertiary structure will broke, and enzyme will be denatured, which loses enzymes activity[34].

The ΔH^* value of ADA in patients and control groups was positive and this point to enzyme reaction is endothermic and need energy for the formation of [ES*] complex. The ΔG^* was a positive value, this indicates that the reaction of ES formation is anon spontaneous, nevertheless needs energy therefore called endergonic reaction. The ΔG^* value of this reaction is independent on molecular pathway of mechanism of transformation[35]. The ΔS^* value of ADA was negative, so indicate that the complex [ES*] more arranged than enzyme, wherever the negative value of ΔS^* reverse through increasing the structure arrangement[35].

In conclusion ,the reduction of ADA and AMPDA activities could cause a state of immune suppression, also the increase in NSMCE2 may play a role in development of DNA damage and inflammation disorders in the patients with kidney stones. The increase in NSMCE2 and the decrease in ADA and AMPDA activities could be as a good indicator for complication of renal calculi.

Conflict of interest statement

We declare that we have no conflict of interest.

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