Contents lists available at ScienceDirect

IF: 0.926

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60157-7

Effect of alprostadil combined with Diammonium glycyrrhizinate on renal interstitial fibrosis in SD rats

Wei-Yan Cai[1], You-Yi Gu[2], Ai-Min Li[1*], Huan-Qin Cui[1], Yi Yao[1]

[1]*Department of Pediatrics Internal Medicine, Yuhuangding Hospital, Yantai, Shandong 264000, China*
[2]*Department of Integrated TCM & Western Medicine, Yuhuangding Hospital, Yantai, Shandong 264000, China*

ARTICLE INFO

Article history:
Received 24 August 2014
Received in revised form 10 September 2014
Accepted 15 October 2014
Available online 20 November 2014

Keywords:
Alprostadil
Diammonium glycyrrhizinate
Renal interstitial fibrosis
Protection

ABSTRACT

Objective: To observe effect of alprostadil combined with Diammonium glycyrrhizinate on renal interstitial fibrosis in SD rats. **Methods:** A total of 75 SD rats were randomly divided into A, B, C, D, E groups with 15 in each group. Rats in group A served as the control group received just only but tissue separation without modeling operation, while model of unilateral ureteral obstruction (UUO) was established in B, C, D, E groups. Rats in A, B group were given saline lavage placebo treatment, while rats in C, D, E groups were given diammonium glycyrrhizinate and alprostadil injection. Five rats were sacrificed 1, 2, 3 weeks after modeling, serum creatinine level of femoral venous blood was determined. Transforming growth factor - β 1 (TGF - β 1) and concentration of connective tissue growth factor (CTGF) were also detected by using ELISA. Line renal interstitial tissue was taken after HE staining, renal interstitial TGF - β 1 and CTGF expression were detected by using immunohistochemical method. **Results:** Serum creatinine levels of B, C, D, E group at different time points in were significantly higher than that of group A (P<0.05); serum creatinine levels in group B were significantly higher than that of C, D, E group at each time point (P<0.05). Serum creatinine level of Group E was significantly lower than C, D group after 2, 3 weeks (P<0.05). Rats in A group at each time point showed no significant changes in TGF - β 1 and CREA concentration in serum and kidney tissues (P>0.05); while serum and kidney tissue TGF - β 1, concentration of CREA, expression of rats in B, C, D, E groups showed a gradual increasing trend over time. TGF - β 1 and CREF of Group B in serum and kidney tissues at each time point were significantly higher than that of the other groups (P<0.05). TGF - β 1 and CREF of Group E in serum and kidney tissues at each time point were significantly lower than that of B, C, D group at all time points in serum and kidney tissues (P<0.05). **Conclusions:** Alprostadil combined with diammonium glycyrrhizinate can significantly lower the expression of TGF - β 1 and CTGF in serum and tissues of SD rat with renal interstitial fibrosis, thus inhibit rat renal interstitial fibrosis process. It has synergy protective effect.

1. Introduction

There is no obvious symptom at early onset of chronic kidney disease, which is known as "invisible killer". It has seriously damage to human body health[1-3]. End-stage renal failure is the advanced stage of chronic kidney disease. Once patients with chronic renal failure were in end-stage of renal failure, they can only be treated with lifelong dialysis or renal transplantation with poor prognosis. It brings serious burden to patients and their family[4]. Glomerular sclerosis and renal interstitial fibrosis are the foundation of chronic renal disease. When it occurs, damaged kidney tissues will start to repair itself. The process is mainly characterized by extracellular matrix deposition and a large number of inflammatory cells infiltration. Accumulation of excessive extracellular matrix can cause scar formation and structure reconstruction of kidney fibrous, which will lead to renal parenchyma and renal damage[5-8]. Studies have shown that[9], in patients with chronic kidney disease, kidney damage and renal

*Corresponding author: Ai-Min Li, Chief Physician, M.D., Department of Pediatrics Internal Medicine, Yuhuangding Hospital, Yantai, Shandong 264000, China.
Tel: 13573577032
E-mail: liaimin1965@163.com
Foundation project: It is supported by Yantai Science and Technology Development Projects (2008142-19).

interstitial involvement levels are positively correlated, and renal interstitial fibrosis plays a decisive role. It is the main pathological basis of chronic renal failure. Therefore, correction of renal damage at early stage can delay the progress of chronic kidney disease. Its clinical research has become a hot spot.

Pathogenesis of renal damage is not fully clear. Studies have shown that[9-11], transforming growth factor β1 - (TGF - β1) and connective tissue growth factor (CTGF) will play important roles in the occurrence and development of renal interstitial injury and chronic kidney disease. To study the effect of alprostadil combined with diammonium glycyrrhizinate on the renal interstitial fibrosis, we established unilateral ureteral obstruction model in SD rats, and treat rats with alprostadil combined with diammonium glycyrrhizinate intervention. Then we observed interstitial expression changes and its concentration of TGF - β1 and CTGF before and after treatment, to provide experimental basis for prevention and control of renal interstitial fibrosis.

2. Materials and methods

2.1. Experimental animals

A total of 75 healthy male SD rats, weighting 180-190 g, provided by the Laboratory Animal Center were selected, They were provided with class II feeding with free access to water and food. After 4 days of breeding, models were established. It followed the related provisions of the Regulations on of Experimental Animals.

2.2. Instrument and reagent

DXC800 automatic biochemistry analyzer (BECKMAN Company, USA); Automatic dewaterer (LEICA Company, Germany); Low temperature high speed centrifuge, optical microscope (OLYMPUS Company, Japan). 10% chloral hydrate, provided by the National Medicine Group Chemical Reagent Beijing Co., LTD.; alprostadil (Beijing Ted Pharmaceutical Co., LTD., production, specifications: 10 μg/kit); diammonium glycyrrhizinate injection (Jiangsu Zhengda Pharmaceutical Co., LTD. specifications 50 mg/kit); TGF-β1 single antibody, Goat anti Human CTGF IgG, CTGF ELISA kit and TGF-β1 ELISA kit were from ShangBai Biomedical Technology Co., LTD. Goat anti Rabbit IgG, SP kits, pig anti sheep IgG were from Huamei Biological Engineering Co., LTD.

2.3. Model establishment

Rats were intraperitoneal injected with ketamine anesthesia (120 mg/kg), and fixed on right side. They were sheared, regular disinfected and received abdomen midline incision. They were cut step by step, layers of tissue were separated. After the bladder was exposed, the left ureter was separated, the lower ureteral ducts were ligated with a 5-0 ligation thread, with the upper point at the end of left kidney. Ureteral duct was clipped between ligation points, then incisions were sew.

2.4. Grouping

A total of 75 SD rats were randomly divided into A, B, C, D, E groups with 15 in each group. Group A served as the control group, rats had only tissue separation without modeling operation; while model of unilateral ureteral obstruction (UUO) was established in B, C, D, E group. As the model group, rats in group B were only given comfort saline 10 mL lavage placebo treatment after modeling. Rats in C, D, E group were given diammonium glycyrrhizinate 20 mg/kg^{-1}·d^{-1}, alprostadil 4 μg/kg^{-1}·d^{-1} and combination injection therapy of diammonium glycyrrhizinate and alprostadil 4 μg/kg^{-1}·d^{-1} with same dose as above.

2.5. Serum concentration of TGF - β1, CREA

Five rats were sacrificed 1, 2, 3 weeks after modeling, serum creatinine level of femoral venous blood were determined. TGF-β1 and concentration of connective tissue growth factor (CTGF) were detected by using ELISA.

2.6. Histological observation

After the completion of blood extraction, the left kidney was immediately extracted. It was avaged with warm saline. After fat capsule and renal capsule stripping, about 3 mm^3 kidney tissues were taken along the long axis of kidney. They were fixed in 10% formaldehyde, received conventional dehydration. Then they had paraffin embedding after 24 h, 4 μm tissue section was prepared for HE stain. Expression of TGF - β 1, CREA in renal tissue were observed under microscope. Dyeing method was as follows: they had dewaxing hydration, then were washed by PBS three times, added with 3% H_2O_2 for 10 min, washed by PBS three times. They were heated by microwave for 10 min, added with goat serum for 20 min, and 1:100 rabbit anti human TGF-β1 (primary antibodies), then placed for 1 h at 37 ℃, washed by PBS three times. They were added with 1:60 goat anti rabbit (second antibody), placed for 1 h at 37 ℃, washed by PBS three times. They were added with SP reagent for 30 min at room temperature, washed with PBS, DAB stained and had hematoxylin redyeing, followed by dehydration, transparentation, sealing and then were observed under

microscopy. CTGF was determined by immunohistochemical SP method.

2.7. Statistical analysis

Processed data were analyzed by using SPSS 16.0 software, One-Way ANOVA was used for data comparison among groups (mean±sd), $P<0.05$ was regarded as statistically significant difference.

3. Results

3.1. Compression of serum creatinine levels

Serum creatinine levels of B, C, D, E group at different time points were significantly higher than that of group A ($P<0.05$); serum creatinine levels of group B were significantly higher than those of C, D, E group at each time point ($P<0.05$); serum creatinine level of group E was significantly lower than those of C, D group after 2, 3 weeks ($P<0.05$), as shown in Table 1.

3.2. TGF – β 1 and CREA concentration in serum at each time point

Rats in group A at each time point showed no significant changes in TGF – β 1 and CREA concentration of serum ($P>0.05$); in B, C, D, E groups serum TGF – β 1, concentration of CREA, expression showed a gradual increasing trend over time; TGF – β 1 and CREF of group B in serum at each time point were significantly higher than that of the other groups ($P<0.05$); TGF – β 1 and CREF of group E in serum at each time point were significantly lower than that of B, C, D group at all time points in serum ($P<0.05$), as shown in Table 2.

3.3. Histological observation

Group A showed no or mild expression of TGF- β 1 in renal interstitial tissue, in group B there were large expressions in renal interstitial tissue with increasing trend over time, and both expressions were positively correlated. Expression of TGF- β 1 and CREF in renal interstitial tissue of C, D, E group were significantly lower than that of group B, expression of TGF- β 1 and CREF renal interstitial tissue of group E was significantly lower than that of group C and D (Figure 1, 2).

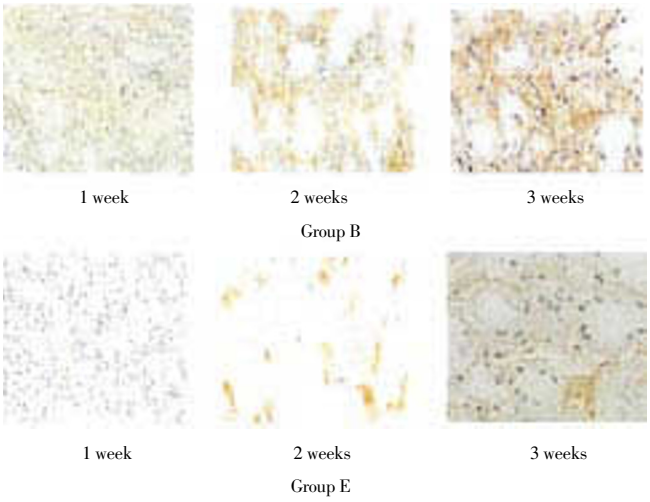

Figure 1. Expression of TGF- β 1 in renal interstitial tissues in group B and E at each time point (×200).

Table 1
Compression of serum creatinine levels (μ mol/L, n=5)

Groups	1 week after modeling	2 weeks after modeling	3 weeks after modeling
A group	56.22±0.32	56.17±0.32	56.20±0.26
B group	61.03±1.13*	82.22±1.29*	139.88±1.08*
C group	58. 58±1.27*#	77.72±0.70*#△	127.30±1.03*#△
D group	56.96±0.62*#	73.08±0.34*#△	117.82±1.19*#△
E group	56.76±0.42*#	66.75±0.94*#	90.72±0.72*#

Compared with group A*$P<0.05$; Compared with group B#$P<0.05$; Compared with group E△$P<0.05$.

Table 2
TGF – β 1 and CREA concentration in serum at each time point.

Groups	TGF- β 1(ng/ mL)			CREA(pg/mL)		
	1 week after modeling	2 weeks after modeling	3 weeks after modeling	1 week after modeling	2 weeks after modeling	3 weeks after modeling
A group	33.06±0.48	32.85±0.15	32.93±0.54	104.87±0.35	105.30±0.32	104.97±0.22
B group	62.30±2.65*	104.40±3.30*	142.24±8.02*	147.22±5.62*	183.92±4.70*	366.40±3.69*
C group	51.20±2.69*#△	94.07±3.28*#△	117.51±5.12*#△	129.93±2.26*#△	144.25±3.90*#△	246.26±4.46*#△
D group	48.18±2.42*#△	84.40±2.27*#△	106.90±3.54*#△	135.02±3.70*#△	156.75±2.67*#△	284.13±4.82*#△
E group	42.09±1.83*#	71.61±2.44*#	90.17±2.37*#	114.55±3.29*#	125.80±4.15*#	185.00±4.07*#

Compared with group A*$P<0.05$; Compared with group B#$P<0.05$; Compared with group E △$P<0.05$.

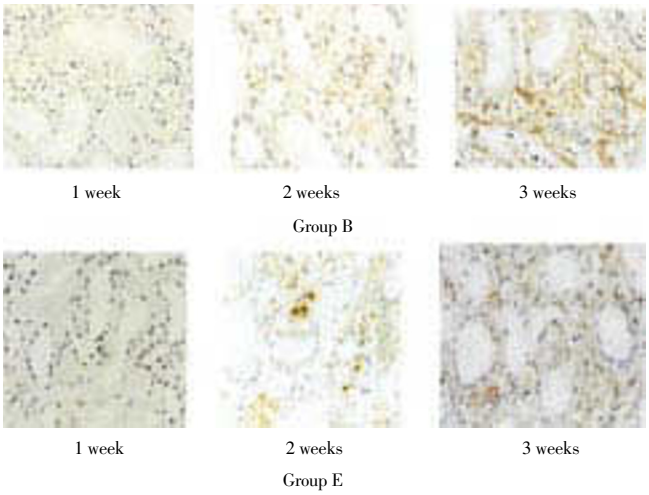

Figure 2. Expression of CREA in renal interstitial tissues in group B and E at each time point (×200).

3.4. TGF – β 1 and CREA concentration in tissues at each time point

Group A of rats at each time point showed no significant changes IN TGF – β 1 and CREA concentration OF tissues (P>0.05); B, C, D, E groups of rats tissues TGF – β 1, concentration of CREA, expression showed gradual increasing trend over time; TGF – β 1 and CREF of Group B in tissues at each time point were significantly higher than that of the other groups (P<0.05); TGF – β 1 and CREF of Group E in tissues at each time point were significantly lower than that of B, C, D group at all time points in tissues (P<0.05), as shown in Table 3.

4. Discussion

Renal interstitial fibrosis happens when repairing damage process is out of control[12]. Renal interstitial fibrosis process may promote/suppress fibrosis cytokine imbalance, oxidative stress, inflammation, *etc*[13]. Renal interstitial fibrosis is common pathway related to a variety of chronic progressive kidney diseases, progressive development is the main pathological basis of kidney failure, and the degree of renal interstitial fibrosis also plays a leading role in the in kidney disease outcome[14-18]. Therefore, the study on renal interstitial fibrosis is of great significance to to delay the progress of chronic kidney disease and improve the prognosis of patients.

Renal interstitial fibrosis pathogenesis is not fully clear. Studies have confirmed that[19-22], serum creatinine level and the degree of renal interstitial injury were positively correlated. In this study, serum creatinine levels of B, C, D, E group at different time points were significantly higher than that of group A, and serum creatinine level of group E was significantly lower than C, D group after 2, 3 weeks of modeling (P<0.05), suggesting alprostadil combined with diammonium glycyrrhizinate can improve renal interstitial fibrosis of the rat kidney function, increase the serum creatinine clearance. Other studies reported the TGF-β 1 and CREA are the basis of renal interstitial fibrosis disease, play an important role in the onset process[23-25]. Therefore, how to inhibit the effect of two kinds of cytokines have become the important way for the treatment of renal interstitial fibrosis. TGF-β 1 is recognized as the strongest fibrosis induced factor. It can induce renal tubular epithelial cells apoptosis, thus promote renal interstitial ECM protein deposition, and cause renal interstitial fibrosis. Studies have shown that[19], glycyrrhizic acid can inhibit the synthesis effectsof prostaglandin E_2 direct on cell membrane, playing the role like glucocorticoid, then to realize the anti fibrosis and to suppress the immune function. In addition, glycyrrhizic acid can inhibit the activity of mesenchymal cells, thus reduce the secretion of TGF-β 1. In this study, renal interstitial TGF-β 1 expression and concentration in serum of group E was significantly lower than that of B, C, D group (P<0.05), suggesting alprostadil combined with diammonium glycyrrhizinate can significantly inhibit renal interstitial fibrosis in the rat TGF-β 1, CREA expression, so as to delay the process of renal interstitial fibrosis. This study showed that, Group A showed no or mild expression of TGF-β 1 in renal interstitial tissue. In group B there were large expressions in renal interstitial tissue with increasing trend over time, and both expressions were positively correlated. Expression rates of TGF-β 1 and CREF renal interstitial tissue of C, D, E group were significantly lower than that of group B, expression rate of TGF-β 1 and CREF renal interstitial tissue of group E was significantly lower

Table 3
TGF – β 1 and CREA concentration in tissues at each time point (%,n=5).

Groups	TGF-β 1			CREA		
	1 week after modeling	2 weeks after modeling	3 weeks after modeling	1 week after modeling	2 weeks after modeling	3 weeks after modeling
A group	3.05±0.12	3.06±0.09	3.06±0.10	0.97±0.18	0.98±0.16	0.97±0.24
B group	11.03±0.24*	16.47±0.48*	34.88±0.59*	3.27±0.42*	7.00±0.45*	14.73±0.75*
C group	7.63±0.46$^{*\#\triangle}$	12.90±0.50$^{*\#\triangle}$	22.31±0.75$^{*\#\triangle}$	1.96±0.13$^{*\#\triangle}$	4.04±0.34$^{*\#\triangle}$	9.06±0.63$^{*\#\triangle}$
D group	8.83±0.44$^{*\#\triangle}$	13.95±0.13$^{*\#\triangle}$	28.94±1.48$^{*\#\triangle}$	2.75±0.30$^{*\#\triangle}$	5.75±0.32$^{*\#\triangle}$	11.30±0.62$^{*\#\triangle}$
E group	5.73±0.52$^{*\#}$	9.70±0.66$^{*\#}$	15.31±0.75$^{*\#}$	1.50±0.22$^{*\#}$	3.54±0.38$^{*\#}$	6.55±0.47$^{*\#}$

Compared with group A$^{*}P$<0.05; Compared with group B$^{\#}P$<0.05; Compared with groupE $^{\triangle}P$<0.05.

than that of group C and D, suggesting that alprostadil combined with diammonium glycyrrhizinate can effectively alleviate the renal fibrosis, improve renal function. Their synergy mechanism may be related to downgrading of TGF-β1 and CTGF in the renal interstitial expression.

Alprostadil combined with diammonium glycyrrhizinate can significantly lower the expression of TGF-β1 and CTGF in serum and tissues of SD rat with renal interstitial fibrosis, thus inhibit rat renal interstitial fibrosis process. They have synergy protective effect on renal interstitial fibrosis in SD rats.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Koesters R, Kaissling B, Lehir M, Picard N, Theilig F, Gebhardt R, et al. Tubular overexpression of transforming growth factor-pi induces autophagy and fibrosis but not mesenchymal transition of renal epithelial cells. *Am J Pathol* 2010; **177**(2): 632-643.

[2] Wang XB, Feng Y, Wang N, Cheung F, Wong CW. Recent progress on anti-liver fibrosis candidates in patents of herbal medicinal products. *Recent Pat Food Nutr Agric* 2012; **4**(2): 91-106.

[3] Huang L, Qi LY, Chen ZL, Li YL, Wen ZY. The compound anti liver fibrosis prescription optimization. *J Southern Med Univ* 2012; **32**(1): 106-108.

[4] Guo LY, Hu SX. Alprostadil effects of prostavasin on apoptosis by renal ischemia-reperfusion injury in rabbits. *Chin J Experimental Surg* 2011; **28**(8): 1356-1358.

[5] Peng WP, Huang YZ, Huang DP. Kay joint salvia miltiorrhiza injection with polyphenols acid salt injection treatment of chronic renal failure: clinical observation on 29 cases. *J Hainan Med Coll* 2010; **21**(5): 33-34.

[6] Cho ME, Kopp JB. Pirfenidone: an anti-fibrotic therapy for progressive kidney disease. *Expert Opin Lnvestiq Druqs* 2010; **19**(2): 275-283.

[7] Andrew L. CCN2: a bona fide target for anti-fibrotic drug inter-vention. *Cell Commun Signal* 2011; **5**: 131-133.

[8] Liao SX, Zhang HY, Gao HC, Li XK. Preliminary study of the treatment of renal fibrosis in rats. *J Wenzhou Med Coll* 2011; **41**(3): 217-220.

[9] Wang YX, Liu SJ, Fu TH, Wang H, Nie B, Sun WW, Liu LY, et al. Renal collaterals miniature remove lump three states theory analysis. *J Beijing Univ Trad Chin Med* 2010; **17**(3): 17-18.

[10] Yang J, Wang C, Shao MH, Qin JY, Wu D, Li RL, et al. Effect and mechanism of renal failure number Ⅱ party 5/6 on nephrectomy rat renal blood flow and oxygen consumption. *Chin J Trad Chin Western Med Comb Kidney Dis* 2011; (12): 578-581.

[11] Rafiq K, Hitomi H, Nakano D, Nishiyama A. Pathophysiological roles of al-dosterone and mineralocorticoid receptor in the kidney. *J Pharmacol Sci* 2011; **115**(1): 1-7.

[12] Fukuda S, Horimai C, Harada K, Wakamatsu T, Fukasawa H, Muto S, et al. Aldosterone- induced kidney injury is mediated by NF κ B activation. *Clin Exp Nephrol* 2011; **15**(1): 41-49.

[13] Fu WC, Wang H, Wang YM, Liu YJ, Peng W. Curcumin joint spironolactone improve aldosterone induced transdifferentiation of renal tubular epithelial cells in rats. *Chin J Trad Chin Western Med Comb Kidney Dis* 2011; **12**(6): 478-481.

[14] Soetikno V, Sari FR, Veeraveedu PT, Thandavarayan RA, Harima M, Sukumaran V, et al. Curcumin amelioratesmacrophage infiltration by inhibiting NF- κ B activation and proin-flammatory cytokines in streptozotocin induced- diabetic nephropathy. *Nutr Metab (Lond)* 2011; **8**(1): 35.

[15] Jena S, Chainy GB. Regulation of expression of antioxidant enzymes by vitamin E and curcumin in L-thyroxine-induced oxidative stress in rat renal cortex. *Mol Biol Rep* 2011; **38**(2): 1047-1054.

[16] Fu WC, Peng W, Wang H, Wang YM, Liu YJ. Curcumin protection on aldosterone induced renal interstitial fibrosis. *Mod Comb Trad Chin Western Med Mag* 2013; **22**(2): 133-138.

[17] Rao J, Wu JY, Li KY, Hu X, Jiang YN, Huang XS. Mechanism of chronic renal fibrosis. *J Liaoning Trad Chin Med* 2014; **41**(4): 826-828.

[18] Siragy HM, Carey RM. Role of the intrarenal rennin-angitensin-aldosterone system in chronic kidney disease. *Am J Nephrol* 2010; **31**(6): 541-550.

[19] Zhu SL, Chen JH, Jiang Y, Ye L, Zhu HH, Peng BS. The active vitamin D3 protection for dialysis patients with chronic kidney disease before kidney. *Chin J Trad Chin Western Med Comb Kidney Dis* 2011; **12**(7): 620-623.

[20] Zhu DY, Sun Y, Sun N, Mao LY. Active vitamin D3 inhibits renal interstitial fibrosis in rats UUO model. *Chin J Trad Chin Western Med Comb Kidney Dis* 2013; **14**(3): 207-211.

[21] Shimada M, Yamabe H, Osawa H, Nakamura N, Kumasaka R, Murakami R, et al. Extracellular matrix metallo-proteinase inducer is expressed in the proximal tubular epithelial cells of the human kidney. *Nephrology (Carlton)* 2009; **14**(2): 171-178.

[22] Fukuoka M, Hamasaki M, Koga K, Hayashi H, Aoki M, Kawarabayashi T, et al. Expression patterns of emmprin and monocarboxylate transporter-1 in ovarian epithelial tumors. *Virchows Arch* 2012; **461**(4): 457- 466.

[23] Nakamura K, Kodama J, Hongo A, Hiramatsu Y. Role of emmprin in endometrial cancer. *BMC Cancer* 2012; **12**(1): 191.

[24] Wu J, Ru NY, Zhang Y, Li Y,Wei D,Ren Z,Huang XF, et al. HAb18G/CD147 promotes epithelial-mesenchymal transition through TGF- β signaling and is transcription-ally regulated by Slug. *Oncogene* 2011; **30**(43): 4410-4427.

[25] Yanaba K, Asano Y, Tada Y, Sugaya M, Kadono T, Hamaguchi Y, et al. Increased serum soluble CD147 levels in patients with systemic sclerosis: association with scleroder-ma renal crisis. *Lin Rheumatol* 2012; **31**(5): 835-839.