

## The Effects of Adding Spironolactone to ACEI or ARB on Proteinuria in Type 2 Diabetes

Farzaneh Najafi<sup>1,2</sup>, Bibi Saideh Rezvaninejad<sup>3\*</sup>, Seyed Mohammad Mohammadi<sup>4</sup>

1- Assistant Professor Division of Nephrology, Yazd Diabetes Research Center, Yazd, Iran.

2- Assistant Professor, Division of Nephrology, Department of Internal Medicine, Faculty of medicine, Shaheed Sadoughi University of Medical Sciences and Health Services, Yazd, Iran.

3- Resident of internal medicine, Division of Nephrology, Department of Internal Medicine, Shaheed Sadoughi University of Medical Sciences and Health Services, Yazd, Iran.

4- Assistant Professor of Endocrinology, Yazd Diabetes Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

### \*Correspondence:

Bibi Saideh Rezvaninejad, Resident of internal medicine, Division of Nephrology, Department of Internal Medicine, Shaheed Sadoughi University of Medical Sciences and Health Services, Yazd, Iran.

**Email:** rezvaninejad@ssu.ac.ir

**Tel:** (98) 351 728 0217

**Received:** 5 April 2014

**Accepted:** 12 July 2014

**Published in September 2014**

### Abstract

**Objectives:** Interruption of rennin-angiotensin-aldosterone system has become a leading therapeutic strategy in the treatment of diabetic nephropathy; however, ACEIs and ARBs do not uniformly suppress the rennin-angiotensin-aldosterone system. Plasma aldosterone levels are elevated in a group of patients despite therapy and this phenomenon known as aldosterone escape or aldosterone break through.

**Materials and Methods:** Forty-two type 2 diabetic patients with overt proteinuria were randomized into two groups and according to double-blind case-control study, one group was treated with spironolactone and others were treated with placebo for 4 months. Twenty-four hours urine protein, glomerular filtration rate (GFR) and blood pressure were measured at baseline and after 4 months of treatment. Serum potassium was checked at baseline and one month of treatment.

**Results:** Urine protein decreased in case group by 26.5% at the end of 4 months, but increased in control group by 17.9% ( $P=0.003$ ). GFR did not have any significant change in case group ( $P<0.354$ ), but decreased in case group significantly ( $P<0.001$ ).

**Conclusion:** Our study showed that spironolactone 12.5 mg/day is safe (without hyperkalemia and gynecomastia) and effective to decrease proteinuria in diabetic patient with CKD1-2.

**Keywords:** Diabetic nephropathy, Aldosterone, Spironolactone, Aldosterone escape, Chronic kidney disease.

### Introduction

Diabetes is an increasing concerning health problem in the world (1,2). The increased prevalence of diabetes is related to rapid economic development, improved living standards, an aging population, a westernized life style, obesity and decreased physical activity, and short sleep duration (2).

Diabetic nephropathy is a microvascular

complication of diabetes and is the main cause of end-stage renal disease (ESRD) in United States and a leading concern of morbidity and mortality in diabetic patients (3-9). Proteinuria is a well-defined risk factor for progression of CKD and cardiovascular disorder in diabetes (10-16).

For several decades, suppression of the renin-angiotensin-aldosterone system (RAAS) by

angiotensin-converting-enzyme inhibitors (ACEIs) or angiotensin-receptor blockers (ARBs), has been a focus for renoprotection (17).

In clinical trials of ACEIs and ARBs, plasma aldosterone levels, after an initial decline, have been shown to increase in some patients over the long term. This phenomenon is termed "aldosterone escape" or "aldosterone break through"; the incidence of this phenomenon ranged from 10% over 6 months to 53% over 1 year (18). Aldosterone break through occurs in 20% of patients with CHF and 40% of diabetic nephropathy (19). Aldosterone break through is due to cleaving angiotensin I to angiotensin II by non-ACE enzymes, hyperkalemia, AT2-receptor dependent mechanism, and an increase in ANP levels during ARB or ACEI therapy (20).

There is increasing clinical evidence that aldosterone blockade with mineralocorticoid receptor blockers have strong antiproteinuric effects (19,21). Aldosterone may have an independent role in renal fibrosis through TGF- $\beta$  signaling pathways which upregulate collagen synthesis, downregulate the release of the extracellular matrix metalloproteinase collagenase, and promote fibroblasts proliferation. Aldosterone involves in ROS production in proximal tubular cells and leads to cell apoptosis, and also increases connective tissue growth factor (CTGF) gene and protein synthesis that is a TGF- $\beta$  independent pathway. Aldosterone induces renal expression of proinflammatory cytokines: osteopontin, membrane cofactor protein 1, IL-6 and IL-18; it promotes fibroblast growth and proliferation, and induce renal injury at least in part by enhancing PAI-1 expression; which tips the balance in favor of extracellular matrix accumulation, and eventually promoting renal fibrosis (22).

Recent evidence also indicated that aldosterone inhibition may have additional renoprotective effect in diabetic patients especially who experience aldosterone escape phenomenon due to ACEIs treatment (17,19). Spironolactone prevents diabetic nephropathy

through reduction of CTGF synthesis in type 2 diabetic rats (23).

Recent studies show that glomerular podocyte abnormalities (including functional changes and loss) are features of diabetic nephropathy (24,25). In subtotaly nephrectomized rats, mineralocorticoid blockade provided additional renoprotection over the ACEI. Such benefit was paralleled by changes in podocyte number and morphology and independent of BP (26). Therefore, we have provided a double-blind randomized controlled trial study to show the supplemental effect of spironolactone on proteinuria in type 2 diabetic patients.

## Materials and Methods

Between April 2012 and February 2013, we consecutively enrolled 42 type 2 diabetic patients with nephropathy (proteinuria >300mg/24-hr), who were receiving an ACEI and or ARB for more than one year. The patients entered the study according to inclusion criteria (GFR >60ml/min, BMI <30 kg/m<sup>2</sup> and HbA1c <8.5%). We excluded patient with BP <100/60 mmHg or BP >150/90 mmHg and serum k >5 mg/dl.

The study protocol was approved by the ethics committee of our institution; and informed consent was obtained from the patients. This study was a double-blind case-control trial and for ensuring that randomization is tamper proof, we set up a separate randomized facility that the trial staff was contacted by telephone when an eligible participant was ready to be randomized. The staff member was provided the name and study number of the new participant. This information was recorded and the treatment group was then randomly assigned (according to computer programs). Therefore the patients were randomly managed with placebo or spironolactone 12.5 mg/day. Demographic data, BP, BMI, serum potassium, serum creatinine, HbA1c, GFR and 24-hr urine protein were evaluated at baseline. Serum k was checked again after one month; also, BP, serum k, serum creatinine, HbA1c, GFR and 24-hr urine protein were checked

after 4 months again. We measured GFR according to MDRD formula. FBS was measured by standard method, serum k was measured by Ione selective (ElectroAnalyzer-XD648-Madmehr company) and serum creatinine concentration was assessed by Jafee (Bionic, Farasamed company). 24-hr urine collection was done for measurement of protein and creatinine. Urine protein measurement was made by Esleba solution. HbA1c was measured by HPLC (Adams HA 8160, Arcray Company, Japan).

### Statistical Analysis

Statistical analysis was performed by using statistical package for social sciences (SPSS, version 17). Almost all data expressed as mean±SD. Categorical data were compared by means of chi-square test and continuous variables by means of student t-test. We used Mann-Whitney test to evaluate the changes in urine protein before and after intervention in two study groups. Statistical significance was

defined as p-value less than 0.05.

### Results

A total of 42 patients were enrolled in this study. Baseline characteristics, demographic, laboratory parameters and medication used in our study patients are listed in Table 1. There were no statistically significant differences between the two groups in terms of demographic data, blood pressure, GFR, serum creatinine, serum k, 24-hour urine protein using T-test). As presented in Table 2, there was no difference between the two groups for prescription of losartan, captopril and enalapril; but diltiazem was prescribed more in control group (chi-square test and Fisher's exact test) ( $P$ -value=0.019)

At the end of the study, urine protein decreased in spironolactone group by 26.5%, but increased in control group by 17.9%, ( $P$ =0.003, Figures 1,2).

GFR showed no significant changes in case group ( $P$ =0.35), but decreased in case group

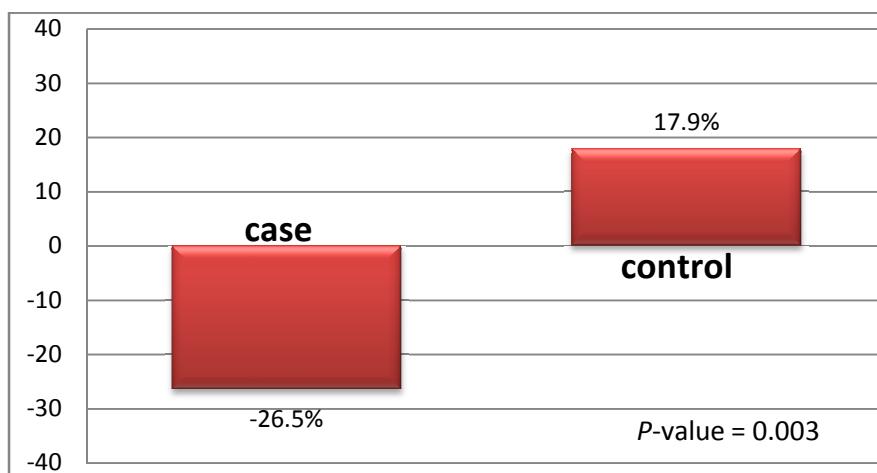
**Table 1. Baseline characteristics of subjects in control and spironolactone group.**

Characteristics	Control group	Spironolactone group	<i>P</i> -value
Age (years)	55.76±10.97	57.19±12.78	0.36
BMI (kg/m <sup>2</sup> )	28.07±1.57	28.29±1.67	0.65
<b>Blood pressure (mmHg)</b>			
Systolic	134.77±10.17	142.23±12.58	0.55
Diastolic	81.50±9.03	82.00±7.18	0.36
HbA1c (%)	7.37±0.82	7.61±0.82	0.88
GFR (ml/min/1.73m <sup>2</sup> )	78.60±12.89	72.9±12.40	0.89
Serum creatinine (mg/ml)	0.99±0.148	1.07±0.173	0.14
Serum potassium (meq/l)	4.64±0.485	4.71±0.444	0.41
24-hr urine protein (mg)	968.09±761.24	1158.90±1534.23	0.20

**Table 2. Comparison of the drugs used for the study groups.**

Medication	Control group	Spironolactone group	<i>P</i> -value
<b>ACEIs</b>			
Enalapril	13 (61.9)	11 (52.8)	0.37
Captopril	5 (23.8)	1 (4.8)	0.09
<b>ARBs</b>			
Losartan	18 (85.7)	20 (95.2)	0.30
Valsartan	2 (9.5)	1 (4.8)	0.50
<b>Calcium channel blocker</b>			
Diltiazem	18 (85.7)	11 (52.4)	0.02

Data are presented as n (%).



**Figure 1.** 24-hr urine protein changes in tow groups before and after intervention.

significantly ( $P < 0.001$ , Figure 3).

Changes in serum potassium, diastolic BP and systolic BP were not significant at the end of study in two groups Figures 4-6.

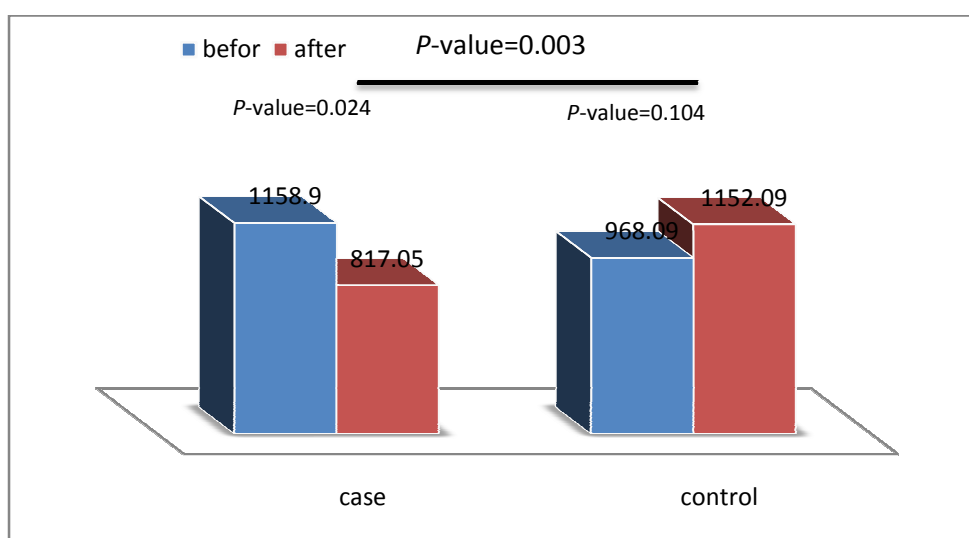
At the end of study, the mean HbA1c in case and control groups was  $7.52 \pm 0.82$  and  $7.28 \pm 0.80$ , respectively, with no significant differences between the two groups ( $P$ -value=0.97).

## Discussion

In this study we showed that treatment with 12.5 mg/day of spironolactone resulted in a 26.5% decrease in urine protein.

In other studies, with use of 25 mg/day of spironolactone, urine protein decreased by 33%, 30%, 15%, 38% and 20%, respectively (27-31). Only in Nowicki study, 12.5mg/day of spironolactone (similar to us) for 4 weeks resulted a decrease in urine protein by 38% (32).

Saklayen et al. did not find any change in serum k with use of spironolactone in diabetic patients, which was in accordance with our findings (33). Also, a systematic review showed that the risk of mineralocorticoid receptor blockade (MRB) –associated hyperkalemia can be minimized if this intervention is reserved for patient with GFR



**Figure 2.** 24-hr urine protein in the two groups before and after intervention. Data are presented as mg/24hr.

greater than 60 ml/min/1.73m<sup>2</sup> (34). In Saklayen study (33), GFR decreased after intervention with spironolactone ( $P=0.0001$ ) while in our study, GFR decreased in control group ( $P=0.001$ ). In a systematic review, MRB therapy (25-50mg/day) was associated with statistically significant decreased GFR in approximately 25% of included studies (34). We did not find any significant changes on systolic or diastolic BPs. Therefore, the effect of spironolactone is independent of decrease in BP. During the study we did not have any gynecomastia in the case group. According to

these, it sounds that spironolactone with a dose of 12.5 mg/day is safe and effective.

### Conclusion

Our study showed that spironolactone 12.5mg/day is safe (without hyperkalemia and gynecomastia) and effective to decrease proteinuria in diabetic patients with CKD1-2. This effect is independent of BP, and can protect GFR. We suggest a large RCT with this dose of spironolactone on CKD 3 and 4.

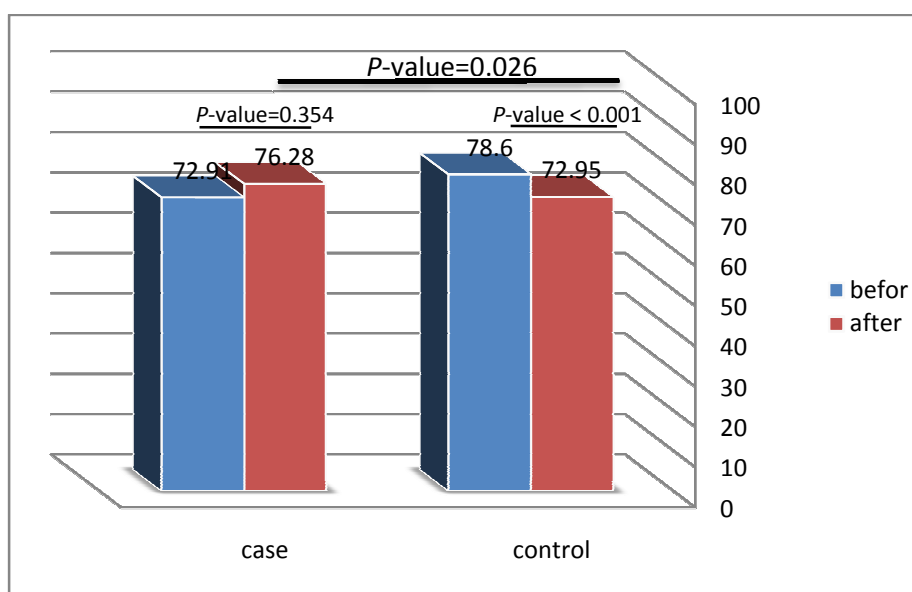


Figure 3. Glomerular Filtration Rate in the two groups before and after intervention. Data are presented as ml/min/1.73m<sup>2</sup>.

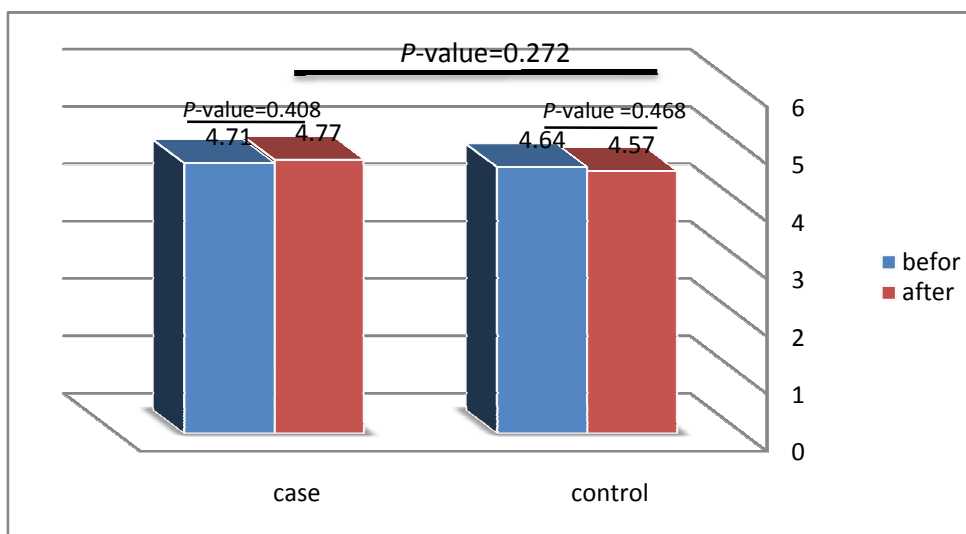


Figure 4. Serum potassium in two groups before and after intervention. Data are presented as meq/l.



## References

- 1- Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes estimates for the year 2000 and projections for 2030. *Diabetes care* 2004;27(5):1047-53.
- 2- Kim DJ. The epidemiology of diabetes in Korea. *Diabetes & metabolism journal* 2011;35(4):303-8.
- 3- Mann JF, Schmieder RE, McQueen M, Dyal L, Schumacher H, Pogue J, et al. Renal outcomes with telmisartan, ramipril, or both, in people at high vascular risk (the ONTARGET study): a multicentre, randomised, double-blind, controlled trial. *The Lancet* 2008;372(9638):547-53.
- 4- Hunsicker LG. Emerging trends for prevention and treatment of diabetic nephropathy: blockade of the RAAS and BP control. *Journal of Managed Care Pharmacy* 2004;10: 12-17.
- 5- Tayebi KH. Short history about renal transplantation program in Iran and the world: Special focus on world kidney day. *Journal of nephropathology* 2012;1(1):5.
- 6- Sarafidis PA, Khosla N, Bakris GL. Antihypertensive therapy in the presence of proteinuria. *American Journal of Kidney Diseases* 2007;49(1):12-26.
- 7- Nasri H, Mortazavi M, Ghorbani A, Shahbazian H, Kheiri S, Baradaran A, et al. Oxford-MEST classification in IgA nephropathy patients: A report from Iran. *Journal of nephropathology* 2012;1(1):31.
- 8- Kidney Disease Outcomes Quality Initiative. K/DOQI clinical practice guidelines on hypertension and antihypertensive agents in chronic kidney disease. *American journal of kidney diseases: the official journal of the National Kidney Foundation* 2004;43(5):S1.
- 9- Einollahi B. Are acquired cystic kidney disease and autosomal dominant polycystic kidney disease risk factors for renal cell carcinoma in kidney transplant patients? *Journal of nephropathology* 2012;1(2):65.
- 10- Burton C, Harris KPG. The role of proteinuria in the progression of chronic renal failure. *American Journal of Kidney Diseases* 1996;27(6):765-75.
- 11- Tolou-Ghamari Z. Nephro and neurotoxicity of calcineurin inhibitors and mechanisms of rejections: A review on tacrolimus and cyclosporin in organ transplantation. *Journal of nephropathology* 2012;1(1):23.
- 12- Khajehdehi P. Turmeric: Reemerging of a neglected Asian traditional remedy. *Journal of nephropathology* 2012;1(1):17.
- 13- Hirschberg R, Wang S. Proteinuria and growth factors in the development of tubulointerstitial injury and scarring in kidney disease. *Current opinion in nephrology and hypertension* 2005;14(1):43-52.
- 14- Magee GM, Bilous RW, Cardwell CR, Hunter SJ, Kee F, Fogarty DG. Is hyperfiltration associated with the future risk of developing diabetic nephropathy? A meta-analysis. *Diabetologia* 2009;52(4):691-7.
- 15- Kadkhodae M. Erythropoietin; bright future and new hopes for an old drug. *Journal of nephropathology* 2012;1(2):81.
- 16- The Diabetes Control and Complications Trial Research Group The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus *N Engl J Med* 1993;329(14):977-86.
- 17- Kang YS1, Ko GJ, Lee MH, Song HK, Han SY, Han KH ,et al .Effect of eplerenone, enalapril and their combination treatment on diabetic nephropathy in type II diabetic rats. *Nephrol Dial Transplant* , 2009;24(1):73-84.
- 18- Bomback AS1, Klemmer PJ. The incidence and implications of aldosterone breakthrough. *Nat ClinPractNephrol.* 2007;3(9):486-92.
- 19- Rossing K1, Schjoedt KJ, Smidt UM, Boomsma F, Parving HH. Beneficial effects of adding spironolactone to recommended antihypertensive treatment in diabetic nephropathy: a randomized, double-masked, cross-over study. *Diabetes Care.* 2005;28(9):2106-12.
- 20- Lars Christian Rump. Secondary rise of albuminuria under AT1-receptor blockade-what is the potential role of aldosterone escape? *Nephrology Dialysis Transplantation* 2014;22(1):5-8.
- 21- Nishiyama A, Kobori H, Konishi Y, Morikawa T, Maeda I, Okumura M, et al. Mineralocorticoid receptor blockade enhances the antiproteinuric effect of an angiotensin II blocker through inhibiting podocyte injury in type 2 diabetic rats. *Journal of Pharmacology and Experimental Therapeutics* 2010;332(3):1072-80.
- 22- Remuzzi G, Cattaneo D, Perico N. The aggravating mechanisms of aldosterone on kidney fibrosis. *Journal of the American Society of Nephrology* 2008;19(8):1459-62.
- 23- Han KH, Kang YS, Han SY, Jee YH, Lee MH, Han JY, et al .Spironolactone ameliorates renal injury and connective tissue growth factor expression in type II diabetic rats. *Kidney international* 2006;70(1):111-20.
- 24- Wolf G, Chen S, Ziyadeh FN. From the periphery of the glomerular capillary wall toward the center of disease podocyte injury comes of age in diabetic nephropathy. *Diabetes* 2005;54(6):1626-34.
- 25- Jefferson JA, Shankland SJ, Pichler RH. Proteinuria in diabetic kidney disease: a mechanistic viewpoint. *Kidney international* 2008;74(1):22-36.

- 26- Nemeth Z, Kokeny G, Godo M, Muzes Ms, Rosivall L, Gross ML, et al. Increased renoprotection with ACE inhibitor plus aldosterone antagonist as compared to monotherapy: the effect on podocytes. *Nephrology Dialysis Transplantation* 2009;24(12):3640-51.
- 27- Schjoedt KJ, Rossing K, Juhl TR, Boomsma F, Rossing P, Tarnow L, et al. Beneficial impact of spironolactone in diabetic nephropathy. *Kidney international* 2005;68(6):2829-36.
- 28- Schjoedt KJ, Rossing K, Juhl TR, Boomsma F, Tarnow L, Rossing P, et al. Beneficial impact of spironolactone on nephrotic range albuminuria in diabetic nephropathy. *Kidney international* 2006;70(3):536-42.
- 29- Nitta K, Uchida K, Nihei H. Spironolactone and angiotensin receptor blocker in nondiabetic renal diseases. *The American journal of medicine* 2004;117(6):444-5.
- 30- Sato A, Hayashi K, Saruta T. Antiproteinuric effects of mineralocorticoid receptor blockade in patients with chronic renal disease. *American journal of hypertension* 2005;18(1):44-9.
- 31- Shiigai T, Kuwana H, Kobayashi T, Maeda Y. Effect of spironolactone added to angiotensin receptor blocker in renal failure patients. *J Am SocNephrol*. 2003;14:763A;(abstr).
- 32- Nowicki M, Muskala P, Bald E, Chwatko G. Nephroprotective effect of combined converting enzyme and aldosterone blockade in hypertensive patients with target organ damage is blood pressure-dependent. *J Am SocNephrol*. 2003;14:21A;(abstr).
- 33- Saklayen MG, Gyebi LK, Tasosa J, Yap J. Effects of additive therapy with spironolactone on proteinuria in diabetic patients already on ACE inhibitor or ARB therapy: results of a randomized, placebo-controlled, double-blind, crossover trial. *Journal of Investigative Medicine* 2008;56(4):714-9.
- 34- Bomback AS, Kshirsagar AV, Amamoo MA, Klemmer PJ. Change in proteinuria after adding aldosterone blockers to ACE inhibitors or angiotensin receptor blockers in CKD: a systematic review. *American Journal of Kidney Diseases* 2008;51(2):199-211.