

Random spot urine protein to creatinine ratio is a reliable measure of proteinuria in lupus nephritis in Koreans

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Abstract

Objectives

The accurate assessment of proteinuria is critical for the management of lupus nephritis. Measuring the protein to creatinine (P/C) ratio in random spot urine (RSU) samples has been introduced as an alternative to the 24-hour (24h) urine collection method. However, it remains unclear as to whether the RSU P/C ratio is reliable for assessing lupus nephritis (LN) in routine clinical practice.

Methods

In total, 275 pairs of 24h urine and RSU samples from 102 patients with biopsy-proven LN were analysed. The correlation and concordance between the P/C ratios in the two sample types were assessed by Pearson or Spearman correlation and intra-class correlation coefficient (ICC) using mixed models for repeated measurements, respectively.

Results

The mean 24h urine P/C ratio was 3.2 ± 4.9 . Overall, RSU P/C ratio correlated strongly with the 24h urine P/C ratio ($r=0.944$, $p<0.001$) with an excellent agreement (ICC=0.949, 95% confidence interval [CI]: 0.69–1.00). Subgroup analyses revealed that the correlation remained high in class II, III, IV, and V LN ($\rho=0.868$, $p<0.001$; $\rho=0.649$, $p=0.007$; $r=0.945$, $p<0.001$; and $\rho=0.900$, $p=0.001$, respectively). The correlation between the 24h urine and RSU P/C ratio in the range of 0.5 to 3 was good ($r=0.720$, $p<0.001$) with ICC of 0.659 (95%CI 0.554–0.812). RSU P/C ratio ≥ 0.5 could predict 24h PCR ≥ 0.5 with 91.7% sensitivity and 70.2% specificity, whereas RSU P/C ratio ≥ 1.0 increased specificity up to 94.7%.

Conclusion

The RSU P/C ratio is an excellent alternative to the 24 hour P/C ratio for assessing the presence of clinically significant proteinuria in LN. RSU P/C ratio >1.0 may prompt directly to a renal biopsy, whereas RSU P/C ratio between 0.5–1.0 should be followed by a confirmatory 24h urine collection.

Key words

proteinuria, lupus nephritis, random spot urine

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Introduction

Systemic lupus erythematosus (SLE) is characterised by a wide range of clinical manifestations (1). Proteinuria is a hallmark of renal diseases and reflects the degree of damage to the glomerular filter system of various aetiologies (2). In systemic lupus erythematosus (SLE), proteinuria correlates with glomerulonephritis activity and thus can help guide diagnostic and therapeutic decisions such as whether to perform a renal biopsy or change the intensity of immunosuppressive treatment (3). Therefore, for the management of lupus nephritis (LN), it is essential that proteinuria is measured accurately.

The gold standard for measuring proteinuria per day, which reflects the damage to glomeruli, is to measure the amount of protein in a 24-hour (24h) urine collection. However, collecting all their urine during a 24h period is a laborious chore for patients. As a result, this method is associated with frequent collection errors that compromise its diagnostic value. To compensate for these errors, the protein levels in 24h samples are now expressed relatively to the creatinine levels, thus yielding the P/C ratio. In the last decade, an alternative to this method was used, namely determination of the P/C ratio in random spot urine (RSU) samples. While this method has been used in various kidney diseases (4, 5), its accuracy in SLE remains unclear (6-9). Our clinical experience over the last two decades suggests that the RSU P/C ratio is a reliable tool that can be used to make clinical decisions, including whether to perform a renal biopsy. To test this notion systematically, a cohort of Korean patients with LN was enrolled and the validity of the RSU P/C ratio as an alternative to 24h urine P/C ratio was examined.

Methods

Patients and sample collections

A total of 275 pairs of 24h urine and RSU samples from 102 Korean patients with a history of biopsy-proven LN who underwent clinical care in Seoul National University Hospital between May, 2004 and August, 2011 were analysed. The study was approved by the

Seoul National University Institutional Review Board.

Creatinine (mg/dL) and protein (mg/dL) levels in the urine samples were measured with a Toshiba-120FR auto-analyser (Toshiba Medical Systems, Tokyo, Japan) and the urine P/C ratio was calculated. The glomerular filtration rate (GFR) was estimated by using the modification of diet in renal disease (MDRD) formula (10). Completeness of the 24h urine collection was assessed by M/E ratio, which compares the total measured creatinine in the samples (M) with the expected creatinine (E). Of note, P/C ratio of 24-hour urine was considered a reliable estimate of 24-hour proteinuria when M/E was 0.5 or more.

Statistical analyses

The normality of continuous variables was assessed by Shapiro-Wilk tests. The correlation between 24h urine P/C ratio and RSU P/C ratio was assessed by Pearson or Spearman correlation, as appropriate. The agreement between RSU and 24h urine P/C ratio was expressed as intra-class correlation coefficient (ICC) using mixed model for repeated measurements. Excellent, good and poor reproducibility were defined as ICC >0.75, 0.4-0.75, or <0.4, respectively (11). All reported *p*-values were two-sided and *p* ≤ 0.05 was considered to indicate statistical significance. Analyses were performed by using IBM SPSS statistics version 19.0 (Chicago, IL, USA) and SAS software (version 9.2; SAS Institute, Cary, NC, USA).

Results

Patient demographics

On average, 2.7 urine samples were obtained from each of 102 patients with a biopsy-proven LN, resulting in 275 pairs of 24h urine and RSU samples. As summarised in Table I, the majority of the patients were women (79.4%). The mean age was 34.0 ± 14.0 years and the mean GFR was 59.3 ± 28.9 mL/min. The mean M/E ratio of 275 collections was 0.99 (±0.36, mean ± SD) and 252 (92%) of 275 24-hour urine collections had M/E ratio >0.5. Of the RSU samples, 56 (20.4%) were from the first morning

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Table I. Characteristics and renal pathology of 102 patients.

Characteristic	Value
Age in years, mean (SD)	34.0 (14.0)
Gender, female	81 (79.4%)
Mean 24h urine P/C ratio (SD)	4.9 (5.1)
GFR (mL/min/1.73m ²) (SD)	59.3 (28.9)
<i>Sample collection</i>	
RSU following the 1 st morning urine	156 (56.7%)
RSU collected at PM	63 (22.9%)
First morning urine	56 (20.4%)
Total	275
<i>Renal pathology, urine sample, n. (%)</i>	
Class I	1 (0.3)
Class II	16 (5.8)
Class III	16 (5.8)
Class III + IV	9 (3.2)
Class III + V	28 (10.2)
Class IV	187 (68.0)
Class IV + V	9 (3.2)
Class V	9 (3.2)

SD: standard deviation; P/C ratio: protein to creatinine ratio; GFR: glomerular filtration rate; RSU: random spot urine.

urine. The remaining 219 RSU samples (79.6%) were from urines produced after the first morning urine.

Strong correlation between 24h urine P/C ratio and RSU P/C ratio

RSU P/C ratio correlated strongly with the 24h urine P/C ratio ($r=0.944$, $p<0.001$) with an excellent agreement

($ICC=0.949$, 95% confidence interval [CI]: 0.69–1.00) (Fig. 1A). The correlation between the 24h urine and RSU P/C ratios in the range of 0.5 to 3 was good ($r=0.720$, $p<0.001$) with ICC of 0.659 (95%CI 0.554–0.812) (Fig.1B).

A RSU P/C ratio ≥ 0.5 could predict 24h PCR ≥ 0.5 with 91.7% sensitivity and 70.2% specificity, whereas RSU P/C ratio ≥ 1.0 detected 24h P/C ratio ≥ 0.5 with 78.9% sensitivity and 94.7% specificity.

Correlation between RSU and 24h urine P/C ratios in different LN classes

The correlation between the RSU and 24h urine P/C ratios in different LN classes was then examined (11). There were 1 class I (0.3%), 16 class II (5.8%), 16 class III (5.8%), 187 class IV (68%), 9 class V (3.2%), 28 class III+V (10.2%), and 9 class IV+V (3.2%) cases (Table I). Subgroup analyses showed that all LN classes except class III had strong correlations between the RSU and 24h urine P/C ratios (Fig. 2): in class III, the correlation was less robust ($\rho=0.649$, $p=0.007$), whereas the correlation remained strong in LN class II ($\rho=0.868$, $p<0.001$) and class IV ($r=0.945$, $p<0.001$). The correlation was also strong in class V ($\rho=0.900$, $p=0.001$). This was also true for class V LN with or without proliferative

changes ($\rho=0.963$, $p<0.001$ for class III+V; $\rho=0.917$, $p<0.001$ for class IV+V).

Stable correlation in a longitudinal follow-up

The stability of these correlations over time was investigated by examining P/C ratios from 46 patients who provided two or more pairs of urine samples at longitudinal follow-up visits. ICC of the 219 pairs was 0.978 (95%CI 0.848–1.0). Among 46 patients, 20 patients had flares. ICC of 134 sample pairs in these 20 patients was 0.904 (95%CI 0.846–0.970) (Fig. 3).

Discussion

The present study showed a strong correlation between the 24h urine P/C ratio and the RSU P/C ratio in LN. These correlations remained robust in different LN classes and over time. LN manifests by proteinuria, active urine sediments, and/or decreased GFR (3) and is the result of damage to the glomerular basement membrane, which regulates glomerular filtration as a highly sensitive and selective filter. However, depending on the extent of damage, proteinuria may be the only sign of active LN (12). Consequently, it is of paramount interest to detect any clinically significant (“threshold”) proteinuria

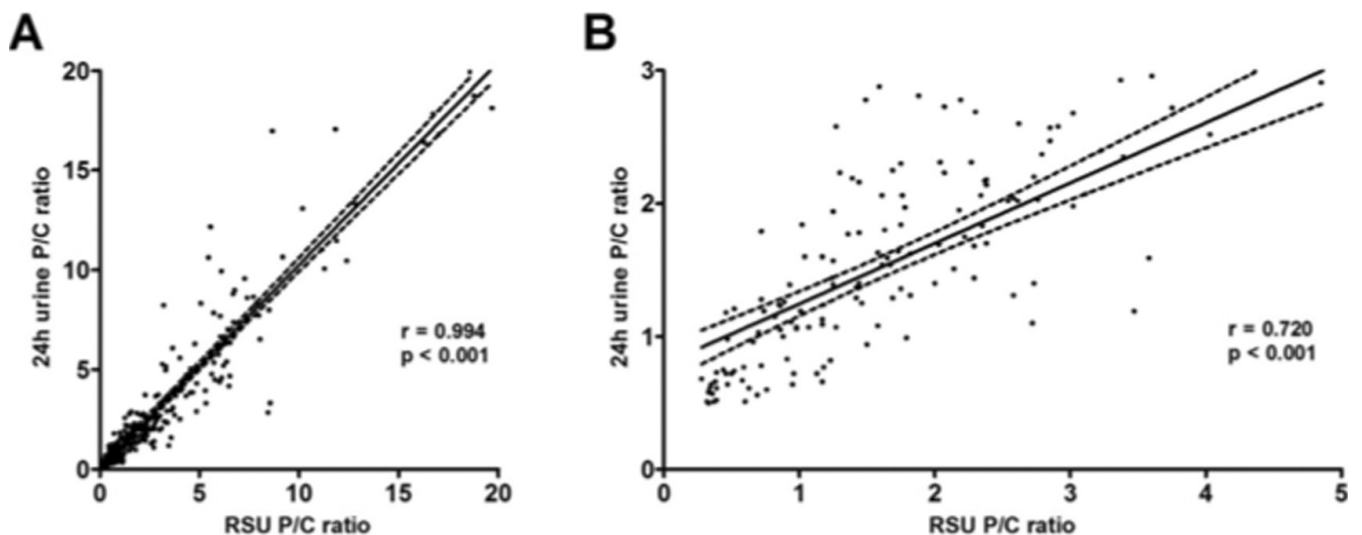


Fig. 1. Correlation between the random spot urine P/C ratio and the 24 hour urine P/C ratio. There was a strong correlation between the 24h urine P/C ratio and the RSU P/C ratio ($n=275$, $r=0.944$, $p<0.001$) with excellent reproducibility ($ICC=0.949$, 95% confidence interval 0.69–1.00) (A). In the low proteinuria range of 0.5–3.0, this correlation became more moderate ($r=0.720$, $p<0.001$) with good reproducibility ($ICC=0.659$, 95% confidence interval 0.554–0.812) (B). Solid lines indicate the linear regression; dotted lines indicate the 95% confidence intervals. RSU: random spot urine; P/C ratio: protein to creatinine ratio.

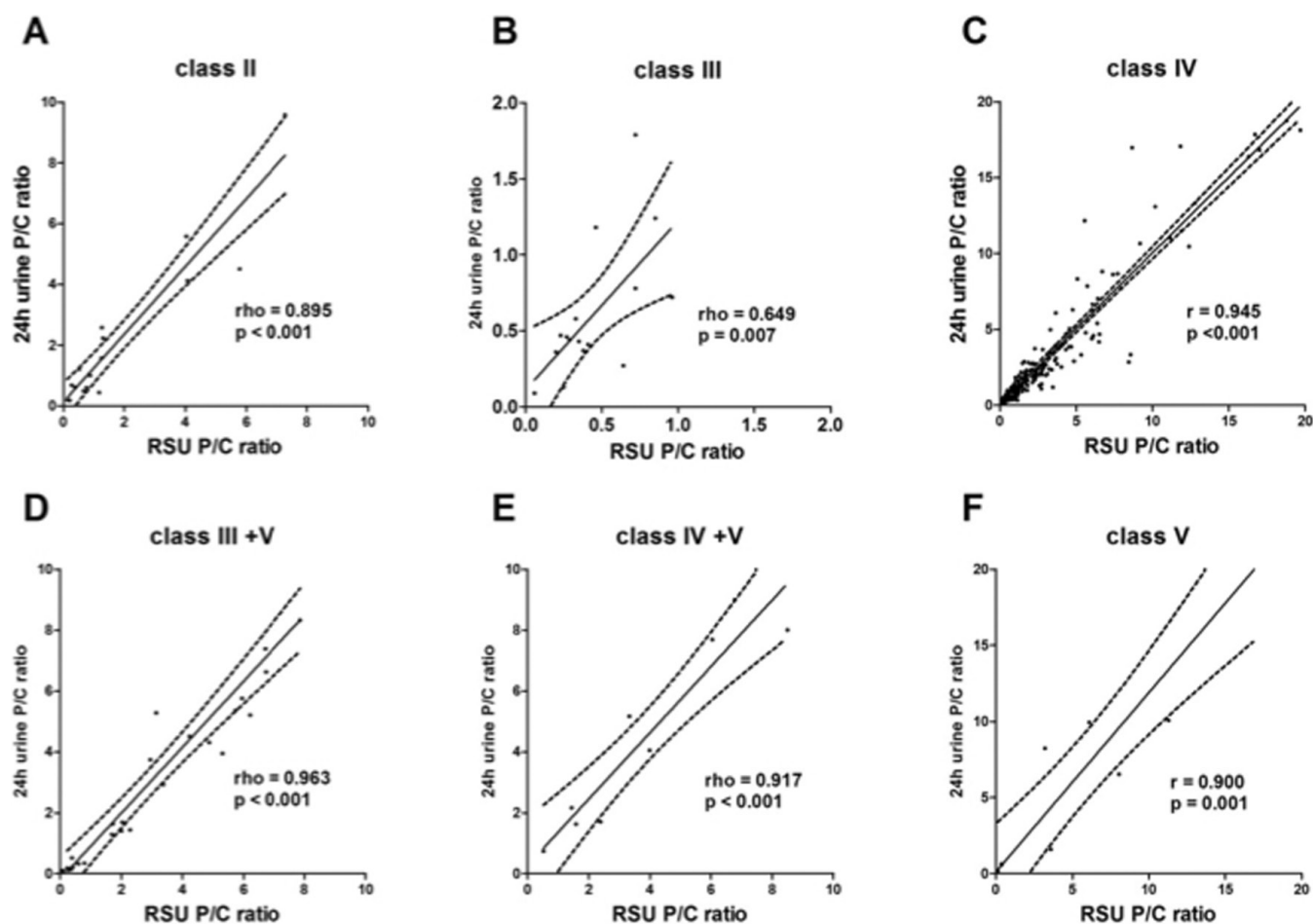


Fig. 2. Correlations between random spot urine P/C ratio and 24 hour urine P/C ratio in different lupus nephritis pathological classes. Class II ($n=16$) showed a strong correlation ($\rho=0.868$, $p<0.001$) (A) but class III ($n=16$) showed a less robust correlation ($\rho=0.649$, $p=0.007$) (B). Class IV ($n=187$) showed a strong correlation ($r=0.945$, $p<0.001$) (C). These strong correlations were also seen in membranous disease, including in class III+V ($n=28$, $\rho=0.963$, $p<0.001$) (D), class IV+V ($n=9$, $\rho=0.917$, $p<0.001$) (E), and class V ($n=9$, $\rho=0.900$, $p=0.001$) (F). Solid lines indicate the linear regressions; dotted lines indicate the 95% confidence intervals. r , Pearson's correlation coefficient; ρ , Spearman's correlation coefficient; P/C ratio, protein to creatinine ratio.

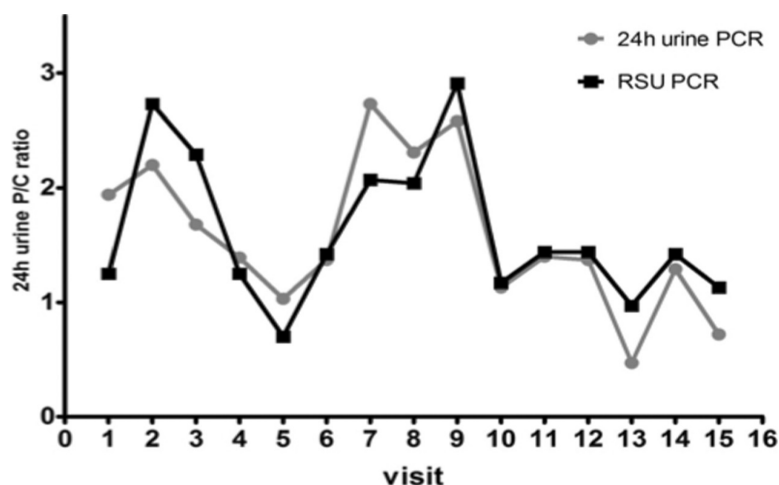


Fig. 3. Correlation between random spot urine P/C ratio and 24-hour urine P/C ratio over time in a representative patient. Patients who provided repeated measures including this representative patient, there was excellent reproducibility (ICC 0.978, 95% confidence interval 0.848–1.0) during the longitudinal follow-up.

≥ 0.5 g per 24h, as this indicates the need for a renal biopsy for the accurate diagnosis and treatment of active LN (13, 14). Since it is nearly impossible to use 24h urine collection as a routine

screening tool in all SLE patients, laboratory assessment such as complete blood count, erythrocyte sedimentation rate, albumin, serum creatinine or GFR, urinalysis and protein/creatinine

ratio (or 24h proteinuria), C3 and C4 are recommended in clinical practice (15, 16). Urine dipstick test detects proteinuria ≥ 0.5 g/day with a sensitivity of 60% or less (17), the RSU P/C

ratio seems to be an ideal screening tool, as it combines the accuracy of 24h urine P/C ratio with the practicability of an urine dipstick. The results of the present study, which examined 275 pairs of 24h and RSU samples, were consistent with those of the studies of Matar *et al.* and Leung *et al.* that found a high agreement between the P/C ratios in 137 and 165 pairs of 24h and RSU samples from patients with LN, respectively (6, 9). However, Birmingham *et al.* reported that spot and 24h urine P/C ratios correlated weakly when the P/C ratios fell into the range of 0.5 to 3.0 (7). Partly consistent with this, the correlation and the concordance were less robust in the P/C ratios range ($r=0.720$, $p<0.001$; ICC=0.659, 95%CI 0.554–0.812) (Fig. 1B).

In a subtle glomerular abnormality, haemodynamic variations during normal daily activity may affect the total amounts of filtered protein; indeed, the proteinuria levels in diverse glomerulopathy and orthostatic proteinuria exhibit circadian variations (18, 19). RSU, which represents a relatively short collection time, is a snap shot and its protein levels may over- or underestimate the average proteinuria over a 24h period. Collection over a longer period of time would smooth those variations out. Supporting this is the report of Fine *et al.*, who found that the P/C ratio of 6-hour timed collections correlated excellently with the P/C ratio of 24h collections and showed high concordance, especially when the urine was collected 0–6 hours after the first voiding urine (20). As above, the RSU P/C ratio >0.5 had a specificity of about 70.2%, *i.e.* overestimating 24h proteinuria in about 30% of cases. The

specificity was increased up to 94.7% when using RSU P/C ratio ≥ 1.0 as the cuff off. As such, we suggest that RSU P/C ratio 0.5–1.0 be followed by a confirmatory 24h urine collection, whereas RSU P/C ratio >1.0 may prompt directly to a renal biopsy.

In conclusion, the RSU P/C ratio is a reliable screening and monitoring tool that can be used to detect clinically significant proteinuria in Korean LN.

References

1. AGMON-LEVIN N, MOSCA M, PETRI M *et al.*: Systemic lupus erythematosus one disease or many? *Autoimmun Rev* 2012; 11: 593-5.
2. BALOW JE: Clinical presentation and monitoring of lupus nephritis. *Lupus* 2005; 14: 25-30.
3. MOSCA M, TANI C, ARINGER M *et al.*: European League Against Rheumatism recommendations for monitoring patients with systemic lupus erythematosus in clinical practice and in observational studies. *Ann Rheum Dis* 2010; 69: 1269-74.
4. LEVEY AS, CORESH J, BALK E *et al.*: National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Int Med* 2003; 139: 137-47.
5. GINSBERG JM, CHANG BS, MATARESE RA, GARELLA S: Use of single voided urine samples to estimate quantitative proteinuria. *New Eng J Med* 1983; 309: 1543-6.
6. LEUNG YY, SZETO CC, TAM LS *et al.*: Urine protein-to-creatinine ratio in an untimed urine collection is a reliable measure of proteinuria in lupus nephritis. *Rheumatology (Oxford)* 2007; 46: 649-52.
7. BIRMINGHAM DJ, ROVIN BH, SHIDHAM G *et al.*: Spot urine protein/creatinine ratios are unreliable estimates of 24 h proteinuria in most systemic lupus erythematosus nephritis flares. *Kidney Int* 2007; 72: 865-70.
8. HEBERT LA, BIRMINGHAM DJ, SHIDHAM G, ROBIN B, NAGARAJA HN, YU CY: Random spot urine protein/creatinine ratio is unreliable for estimating 24-hour proteinuria in individual systemic lupus erythematosus nephritis patients. *Nephron Clin Pract* 2009; 113: c177-82.
9. MATAR HE, PETERSON P, SANGLE S, D'CRUZ DP: Correlation of 24-hour urinary protein quantification with spot urine protein:creatinine ratio in lupus nephritis. *Lupus* 2012; 21: 836-9.
10. LEVEY AS, CORESH J, GREENE T *et al.*: Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Int Med* 2006; 145: 247-54.
11. ROSNER B: Multisample inference. In: *Fundamentals of Biostatistics*. 5th ed. Pacific Grove, CA, Duxbury 2000:511-576.
12. TAN EM, COHEN AS, FRIES JF *et al.*: The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheuma* 1982; 25: 1271-7.
13. CERCERAR, KHAMASHTA MA, FONT J *et al.*: Systemic lupus erythematosus: clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. The European Working Party on Systemic Lupus Erythematosus. *Medicine* 1993; 72: 113-24.
14. BOMBACK AS, APPEL GB: Updates on the treatment of lupus nephritis. *J Am Soc Nephrol* 2010; 21: 2028-35.
15. MOSCA M, TANI C, ARINGER M *et al.*: Development of quality indicators to evaluate the monitoring of SLE patients in routine clinical practice. *Autoimmun Rev* 2011; 10: 383-8.
16. MOON SJ, PARK HS, KWOK SK, JU JH, KIM HY, PARK SH: Predictors of end-stage renal disease and recurrence of lupus activity after initiation of dialysis in patients with lupus nephritis. *Clin Exp Rheumatol* 2013; 31: 31-9.
17. CHOTAYAPORN T, KASITANON N, SUKITAWUT W, LOUTHRENOO W: Comparison of proteinuria determination by urine dipstick, spot urine protein creatinine index, and urine protein 24 hours in lupus patients. *J Clin Rheumatol* 2011; 17: 124-9.
18. SPRINGBERG PD, GARRET LE, JR., THOMPSON AL, JR., COLLINS NF, LORDON RE, ROBINSON RR: Fixed and reproducible orthostatic proteinuria: results of a 20-year follow-up study. *Ann Int Med* 1982; 97: 516-9.
19. KOOPMAN MG, KREDIET RT, ZUYDERHOUDT FJ, DE MOOR EA, ARISZ L: A circadian rhythm of proteinuria in patients with a nephrotic syndrome. *Clin Sci (Lond)* 1985; 69: 395-401.
20. FINE DM, ZIEGENBEIN M, PETRI M *et al.*: A prospective study of protein excretion using short-interval timed urine collections in patients with lupus nephritis. *Kidney Int* 2009; 76: 1284-8.