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Clinical study of the effects of menstrual blood on proteinuria

Natsuki GOTO, Yume NAGAOKA, Rieko CHINA, Kanna WATANABE,
Yoshitaka MIYAOKA, Miho NAGAI, Yoshihiko KANNO

Department of Nephrology, Tokyo Medical University Hospital

Abstract

Background

It is common for the day of urinalysis to coincide with the days of menstruation in adult women. Although the test results from urine samples taken on menstrual days are generally used as reference values, no studies to date have carefully analyzed the effects of menstrual blood on urine protein levels. Therefore, in this study, we compared protein levels in urine samples taken from subjects on their menstrual days with those from non-menstrual days, and discussed the effects of menstrual blood on urine protein levels.

Materials and Methods

A total of 112 women who visited our clinic between October 1, 2003 and June 30, 2019 and underwent urinalysis on both their menstrual and non-menstrual days were included in the study. Factors analyzed were the difference between urine protein level on menstrual days and non-menstrual days, the difference between urine occult blood level and urine protein on menstrual days, and urine protein levels on menstrual days.

Results

The mean age at the time of urinalysis during menstruation was 35±9.2 years, the mean serum creatinine (Cr) level on urinalysis at the first visit was 0.72±0.271 mg/dL, and the mean serum estimated glomerular filtration rate (eGFR) was 83.4±30.31 mL/min/1.73 m^2. The mean interval between urinalysis on menstrual and non-menstrual days or between urinalysis on non-menstrual and menstrual days was 99±82.5 days (absolute value). On menstrual days, 95.5% of the subjects had urinary occult blood of (+) or more, 67.9% had proteinuria of (+) or more, and 67.0% had both occult blood and proteinuria of (+) or more. On the other hand, on non-menstrual days, 42.0% had urinary occult blood of (+) or more, 48.2% had proteinuria of (+) or more, and 30.4% had both occult blood and protein of (+) or more. The mean urine protein level on menstrual days was 0.61±1.00 g/gCr and on non-menstrual days was 0.51±1.00 g/gCr (paired *t*-test, $p< 0.01$), indicating that urine protein levels were significantly higher on menstrual days than on non-menstrual days. There was also a significant positive correlation between the urinary occult blood levels on menstrual days and the difference in urine protein levels between menstrual days and non-menstrual days (Wilcoxon signed-rank test, $p<0.01$), and the urinary occult blood and the urine protein level on menstrual days was also significantly greater than the difference upon qualitative evaluation (Wilcoxon signed-rank test, $p< 0.01$).

Conclusion

The results of our study suggest that the presence of menstrual blood in a urine sample significantly increases urinary occult blood levels and urine protein levels.

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Corresponding author : Yoshihiko Kanno, Department of Nephrology, Tokyo Medical University Hospital, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan
TEL : +81-3-3342-6111 FAX : +81-3-3345-1437 E-mail : kannoyh@tokyo-med.ac.jp

Introduction

Urinalysis is a simple and noninvasive test and urine protein and urine occult blood analyses are effective for the early detection of glomerulonephritis[1)2)]. In general, even if microscopic hematuria is noted on urinalysis, when proteinuria is not detected and patients have no notable symptoms, they do not need post-tests or follow-up[3)4)]. However, positive results of microscopic hematuria and proteinuria on urinalysis are important signs, because such patients may have glomerulonephritis even if there are no symptoms[1)5)6)]. Otherwise, if an asymptomatic patient is found to have microscopic hematuria on urinalysis and further proteinuria is detected, the possibility of false-positive proteinuria must be kept in mind[7)]. Hemoglobinuria is one cause of false-positive results of proteinuria on urinalysis[8)]. Hemoglobinuria results in a high amount of protein in the urine and can result to false-positive results on urinalysis[9)], and a similar phenomenon can also occur in adult women during menstruation. If the urine is contaminated with menstrual blood, this can cause hemoglobinuria, which may contribute to a false-positive urinalysis result. Because menstrual cycles occur about once a month in adult women, it is common for the days of menstruation to coincide with the day of urinalysis, increasing the likelihood of a false-positive result for occult blood on urinalysis[10)]. In addition, caution is required in the evaluation of urinalysis results in adult women, as the presence of menstrual blood in the urine can affect the amount of urinary protein[1)].

In general, there have been many reports on the association between occult blood and protein levels on urinalysis, but to our knowledge, there have been no reports to date on the effects of the presence of menstrual blood on urine protein levels.

In this study, we hence retrospectively investigated the results of urine protein levels in samples taken from women on menstrual and non-menstrual days, to determine the difference in urinary protein levels between menstrual and non-menstrual days, and changes in urinary occult blood parameters.

Materials and methods

This study included 6,881 women who visited our clinic between October 1, 2003 and June 30, 2019, and underwent urinalysis on both menstrual and non-menstrual days. Confirmation of whether the day of the test was a menstrual day or not was based on a self-report by the patients who were eligible for the test. Among the 6,881 patients, those with an interval between urinalysis on menstrual and non-menstrual days or an interval between urinalysis on non-menstrual and menstrual days of more than 18 months, urine protein ≥ 10 g/gCr, urine protein level below detection sensitivity, and serum Cr > 1.5 mg/dL were excluded from the study, and a total of 6,769 patients were excluded. Therefore, urine protein levels on menstrual and non-menstrual days of 112 patients who were available for follow-up were analyzed.

The endpoints that were assessed were the difference between urine protein on menstrual days and urine protein on non-menstrual days, the urine occult blood level on menstrual days and the difference in urine protein between on menstrual days and non-menstrual days, and the urine occult blood level and urine protein on menstrual days. If more than one result for an item was available for a patient during the study period, the result from the most recent analysis was used.

The qualitative test for urine protein level was performed by the test paper method using the automated urine analyzer US-3500Rplus (EIKEN CHEMICAL CO., LTD., Tokyo, Japan). Uropaper αIII EIKEN 9L (EIKEN CHEMICAL CO., LTD.) was used as the test paper.

Urine protein levels were measured by the pyrogallol red coloration method using an automated analyzer Drug-AR Wako Micro TP-AR (FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan), and urinary Cr levels were measured by the L-type Wako CRE-M quantification method (FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan) by the enzymatic method using creatininase and HMMPS reagents. Urine protein was calculated by dividing the urine protein value by the Cr value. The results of the qualitative analysis of urine occult blood, (−), (±), (1+), (2+), and (3 +), were replaced by 1, 2, 3, 4, and 5, respectively, so they could be subjected to statistical analysis[11)12)]. As the rate of a negative proteinuria result was found to be more than 92.3% in male and female adult subjects in Japan who were below G3 of the chronic kidney disease (CKD) classification[13)], the upper limit of serum Cr in this study was set to 1.5 mg/dL, which is the value of eGFR 30 mL/min/1.73 m^2 converted to women in their 20s to 50s. Patients were stratified by their urine protein level, which was calculated as the urine protein to urine Cr ratio (g/gCr).

Data were presented as the mean ± standard deviation and analyzed for significant difference by the paired *t*-test and Wilcoxon signed-rank test (Prism 8, GraphPad Software, San Diego, CA). A *p*-value of less than 0.05 was considered to indicate a statistically significant difference between groups.

This study was approved by the Ethics Committee for Medical Research of Tokyo Medical University, Japan (study registration no. 2017-137), and informed consent was obtained from the patients.

Results

Baseline characteristics of the patients and prevalence of proteinuria and hematuria

The mean age at the time of urinalysis (menstrual day) in the 112 patients who were included in the study was 35 ± 9.2 years, the mean serum Cr level of initial urinalysis was 0.72 ± 0.271 mg/dL, and the mean serum eGFR level was 83.4 ± 30.31 mL/min/1.73 m^2 (Table 1). The mean interval between urinalysis on menstrual and non-menstrual days or between urinalysis on non-menstrual and menstrual days was 99 ± 82.5 days (absolute value). On menstrual days, 95.5% of the subjects had urinary occult blood (+) or more, 67.9% had proteinuria (+) or more, and 67.0% had both occult blood and protein (+) or more. On the other hand, 42.0% of the subjects had (+) or more urinary occult blood, 48.2% had (+) or more proteinuria, and 30.4% had (+) or more both occult blood and protein on non-menstrual days.

Differences in average proteinuria levels between menstrual and non-menstrual days

The average protein level on menstrual days was 0.61 ± 1.00 g/gCr and on non-menstrual days was 0.51 ± 1.00 g/gCr (paired *t*-test, $p<0.01$), and the protein level was significantly higher on menstrual days than on non-menstrual days(Fig. 1).

Associations between occult hematuria and proteinuria levels

There was a significant positive correlation between urinary occult blood level on menstrual days and increase in protein quantification between on menstrual and non-menstrual days (Wilcoxon signed-rank test, $p<0.01$) (Fig. 2). In particular, urine protein level was shown to increase in subjects with occult blood of (2+) or more. In 31 of the 112 patients, the difference in urine protein level was negative, and higher urinary protein values were detected on non-menstrual days than on menstrual days.

There was a significant positive correlation between urine protein level and urinary occult blood level on menstrual days (Wilcoxon signed-rank test, $p<0.01$) (Fig. 3), and the maximum urinary protein level was also found in a patient with a urinary occult blood level of (3+). These results suggest that urinary protein levels were significantly increased in conjunction with an increase in urinary occult blood levels.

Table 1 Baseline characteristics of the study participants and the proportion of participants with proteinuria and hematuria on menstrual days and non-menstrual days.

Mean age（years）	35 ± 9.2	
Body mass index（kg/m^2）	22.7 ± 5.43	
Laboratory data		
eGFR（mL/min/1.73 m^2）	83.4 ± 30.31	
Albumin（g/dL）	4.0 ± 0.48	
Creatinine（mg/dL）	0.72 ± 0.271	
Primary diseases, Comorbid conditions, *n*,（%）		
Hypertension	15（13.4）	
Diabetes mellitus	4（3.5）	
Uterine myoma	3（2.7）	
Renal diseases	74（66.1）	
IgA nephropathy	30（26.8）	
Minimal change nephrotic syndrome	13（11.6）	
Lupus nephritis	13（11.6）	
Others	18（16.1）	
Concurrent medication, *n*,（%）		
Prednisolone	39（34.8）	
Angiotensin II receptor blocker	32（28.6）	
Dipyridamole	9（8.0）	
Dilazep hydrochloride hydrate	5（4.4）	
Eicosapentaenoic acid/ Docosahexaenoic acid	7（6.2）	
	Menstrual day	Non-menstrual day
Proteinuria, *n*,（%）	76（67.9）	54（48.2）
Proteinuria and hematuria, *n*,（%）	75（67.0）	34（30.4）
Hematuria, *n*,（%）	107（95.5）	47（42.0）

Values are shown as the mean ± standard deviation unless otherwise indicated.
eGFR, estimated glomerular filtration rate.

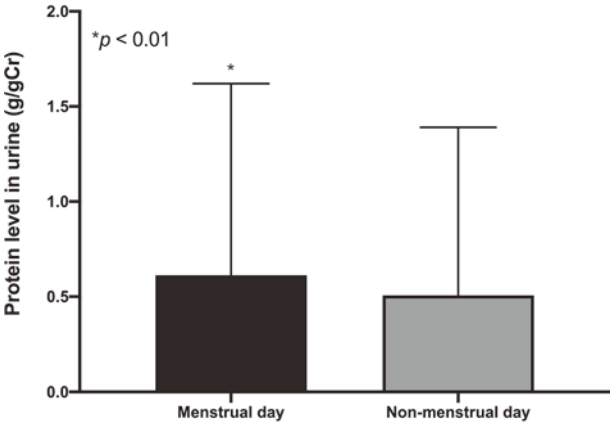

Fig. 1 Average proteinuria levels
The mean protein level on menstrual days was 0.61 ± 1.00 g/gCr and on non-menstrual days was 0.51 ± 1.00 g/gCr. The protein level was significantly higher on menstrual days than on non-menstrual days (p<0.01 ; paired t-test).

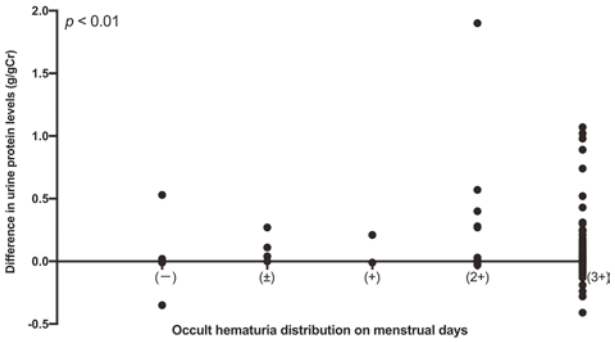

Fig. 2 Difference in proteinuria levels and occult hematuria levels on menstrual days
A significant positive correlation was observed between urine occult blood level on menstrual days and difference in urine protein levels between on menstrual and on non-menstrual days (p<0.01 ; Wilcoxon signed-rank test).

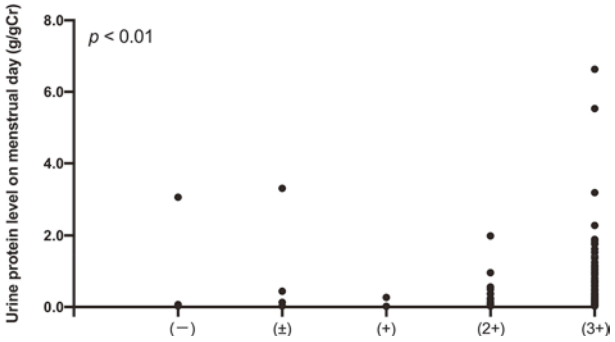

Fig. 3 Distribution of urinary protein and occult hematuria levels on menstrual days
A significant positive correlation was observed between an increase in urinary occult blood level and increase in urinary protein level (p<0.01 ; Wilcoxon signed-rank test).

Discussion

Protein levels in urine collected on menstrual days was significantly higher than those on non-menstrual days in our study. Regarding the criteria for further analysis of urine protein levels, Sakai et al. stated that 0.15 g/gCr or higher is the criteria for the diagnosis of CKD, and referral to a specialist is recommended at 0.50 g/gCr or higher[15]. Recently, proteinuria has been found to be a predictor of long-term mortality after myocardial infarction[16–18], and a predictor of all-cause mortality in the general population[19][20], and hence these studies have demonstrated the clinical role played by proteinuria[21][22]. Although the difference between mean urine protein levels on menstrual and non-menstrual days in the present study was 0.10 g/gCr, which would not result in a substantial effect on the diagnosis or treatment strategy, if possible, conducted urinalysis on non-menstrual days was appropriate. When a urine protein analysis is performed on a menstrual day, our results indicate that it may be useful to keep in mind that urine protein analysis of women on menstrual days may result in the overestimation of urine protein levels by about 0.10 g/gCr.

Furthermore, regarding the association between urinary occult blood and urinary protein levels, urinary protein levels were significantly increased in conjunction with an increase in urinary occult blood levels. On the other hand, 31 of the 112 patients had higher urine protein levels on non-menstrual days than on menstrual days ; however, this may be owing to physiological proteinuria caused by exercise or fever and pyuria due to urinary tract infection[23], leading to increased urinary hemoglobin levels owing to a decrease in urine pH and weakening of the cell membrane of red blood cells[24]. On the other hand, the reason for decreased urine protein levels on menstrual days may be because even if menstrual blood has contaminated to the urine, red blood cells may not disintegrate if the urine is alkaline[8], and medication for a primary disease, such as angiotensin receptor antagonists, which were started between the day of urine sampling on a menstrual day and that on a non-menstrual days may have reduced the level of proteinuria[25]. Therefore, it has been difficult to quantify the increase in urinary occult blood qualitative levels and urinary protein quantitative levels due to the influence of various factors. Another reason for the discrepancy between occult blood and urine protein levels was that the sensitivity and specificity of the urine occult blood test using the test paper method was 75.3% and 88.6%, respectively. This suggests the possibility of false-positive and false-negative results in both subjects with or without diseases presenting with hematuria[14] ; i.e., even if a subject was healthy, proteinuria may be positive in 3% to 5% of all urinalyses in both men and

women, and hematuria and proteinuria may be present in 1% of all urinalyses[10]. Therefore, it was suggested that proteinuria levels may not necessarily correlate with occult blood levels, depending on a patient's underlying disease, oral medication, activity and physical condition on the day before the test, and the condition of the urine sample.

There are several limitations to this study. Although the number of patients included in this study was relatively large, many of the patients who underwent urine protein level analysis at the time of their outpatient consultations had underlying medical conditions, with 66.1% of the patients having renal disease. The study was conducted as a retrospective study at a single hospital, so we were unable to eliminate population bias. In addition, we were unable to clarify whether subjects had exercised on the day before their visit, and whether they had taken any medications that may affect urinary pH and protein concentration, which may subsequently affect urine protein levels. We only investigated the occult blood reaction in the urine occult blood test, and not erythrocyte count, which may have affected myoglobinuria in some of the patients in this study. Although the patients self-reported whether they were menstruating, information regarding the specific day of their menstrual cycle was not obtained, and hence the effects of blood in the urine samples taken from subjects during the beginning or end of their menstrual cycle may have been estimated to be less than that of samples from the middle of the menstrual cycle. In addition, as the subjects were not enforced to collect intermediate urine, some specimens may contain primary urine, which may have higher blood levels.

In conclusion, urine protein levels were increased on menstrual days compared with non-menstrual days, suggesting that urine occult blood levels were increased and urine protein levels were significantly increased by menstrual blood contamination. Future prospective studies on the association of urinary occult blood and urinary protein levels are required to further support the results of this study.

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尿蛋白検査における月経血混入の影響に関する臨床研究

後藤　菜津希　長岡　由女　知名　理絵子
渡邊　カンナ　宮岡　良卓　長井　美穂
菅野　義彦

東京医科大学腎臓内科学分野

【要旨】

はじめに

成人女性において、月経日と尿検査日が重なってしまうことは少なくない。その際、月経日の尿検査結果は参考値として扱われることが一般的であるが、尿蛋白値への影響度を精査した研究において統一された見解はない。そこで今回われわれは、月経日と非月経日を比較し、月経血混入が尿蛋白に与える影響について考察したので報告する。

対象と方法

本研究では 2003 年 10 月 1 日から 2019 年 6 月 30 日までに当院を受診し、月経日および非月経日の両日ともに尿検査を実施した女性 112 例を対象とした。評価項目は、月経日の UP と非月経日の UP との差、月経日における尿潜血定性と UP の差、月経日における UP 値とした。

結果

月経検査実施時の平均年齢は 35±9.2 歳、初診時の尿検査における平均血清 Cr は 0.72±0.271 mg/dl、平均血清 eGFR は 83.4±30.31 ml/min/1.73 m^2 であった。また、月経検査日から非月経検査日までの間隔、もしくは非月経検査日から月経検査日までの間隔は平均 99±82.5 日（絶対値）であった。月経日において尿潜血が（+）以上であった割合は 95.5%、蛋白尿が（+）以上であった割合は 67.9%、潜血および蛋白の両方が（+）以上であった割合は 67.0% であった。一方、非月経日において尿潜血が（+）以上であった割合は 42.0%、蛋白尿が（+）以上であった割合は 48.2%、潜血および蛋白の両方が（+）以上であった割合は 30.4% であった。月経日における尿蛋白定量は平均 0.61±1.00 g/gCr、非月経日における尿蛋白定量は平均 0.51±1.00 g/gCr であり（Paired *t*-test, $p<0.01$）、月経日では非月経日と比較して有意に尿蛋白定量が大きくなることが分かった。また、月経日の尿潜血定性値と、月経日と非月経日における尿蛋白定量値の差において、尿潜血定性値と尿蛋白定量の差に有意な正の相関関係を認め（Wilcoxon signed-rank test, $p<0.01$）、月経日における尿潜血定性値と尿蛋白定量値に関しても、尿潜血定性値と尿蛋白定量値に有意な正の相関関係を認めた（Wilcoxon signed-rank test, $p<0.01$）。

考察

本研究の結果より、月経血混入により尿潜血定性が増加するとともに尿蛋白定量値が有意に増加することが示唆された。

〈キーワード〉　血尿、月経、蛋白尿