#39 Hormone metabolites predict urinary epithelial cell counts during a menstrual cycle in healthy controls

Liou N[1], Kelpie A[2], Chieng C[2], Kong Q[3], Khasriya R[3], Horsley H[2]

1. Bladder Infection and Immunity Group, Institute for Women's Health, University College London, 2. Bladder Infection and Immunity Group, Department of Renal Medicine, University College London, 3. Bladder Infection and Immunity Group, Eastman Dental Institute, University College London

Background

Women are at increased risk of urinary tract infections (UTI).

- There appears to be a **hormonal role** with UTIs often coinciding with menstrual changes, particularly menstrual status e.g. post menopause.
- Premenopausal women are at risk of UTI yet 20% of women with UTI symptoms have infections missed using routine diagnostic tests [1].

Issues with testing:

- **Epithelial cells** found in urine samples are deemed to be contaminated by skin flora, an issue thought to be more common in women, and hence the mid stream urine as the 'gold standard' in urine collection [2].
- Current diagnostic thresholds are set to identify cellular markers of infection, namely white blood cells, while little is known about the physiological effects of reproductive hormone levels on the types of cells found in the urine.

Aim:
To longitudinally assess urinary cells over a menstrual cycle. We hypothesised that the urinary hormonal metabolites of oestrogen and progesterone predict urinary cell counts in healthy premenopausal controls.

Methods

Longitudinal: urine sampling thrice weekly at ± 2 day over menstrual cycle

Inclusion: healthy, premenopausal women, no recent UTI or antibiotics, no lower urinary tract symptoms (LUTS) using 39-point symptom questionnaire

Dipstick: dipsticks and commercially available LH ovulation sticks

Brightfield Microscopy: cell count/µl for white blood cells (WBC), red blood cells (RBC), and epithelial cells (EPC)

Immunofluorescence: DAPI, WGA and uroplakin 3 (UP3), a marker of terminal urothelial differentiation to identify EPC from the urinary tract

ELISA: urinary hormone metabolites estrone-3-glucuronide (E1G) and pregnanediol glucuronide (PDG) which correlate with serum levels [3].

Statistics: Logarithmic transformation was applied to non-parametric results, and linear regression performed using R for statistical computation.

Ethics: REC 11/LO/0109, IRAS ID 67316. **Funding**: philanthropic donation

Results

14 participants recruited, 214 samples over 8 weeks (Table 1-2)
29% on hormonal treatment for contraception or polycystic ovarian syndrome
WBC and EPC present in all samples of asymptomatic participants (Table 3)

Table 1

Demographic	Mean, SD or n, %
Age	28 ± 5 years
BMI	23.3 ± 5.5
White	6, 43%
Asian	5, 36%
Black	2, 14%
Mixed	1, 7%
No hormones	10, 71%
Hormones	4, 29%
Cycle length	28.3 ± 2.\9 days
Bleeding length	5.4 ± 0.9 days

Table 2

Demographic	Mean, SD or n, %
Day time frequency	5 ± 1 void/day
Noctural frequency	0 ± 1 void/night
LUTS score	0 ± 0 symptoms

Table 3

Variable	Median	IQR
E1G	18,4000	7,661 - 41,120
PDG	1,481,000	725,250 - 2,585,500
RBC	0	0 - 6
WBC	10	2 - 18
EPC	14	4 - 30
Hormone pgml, Cell count/µl		

ELISA

E1G and PDG median and IQR (table 2) within normal premenopausal range [2]
E1G and PDG fluctuate in participants with bleeding cycles (Figure 1)

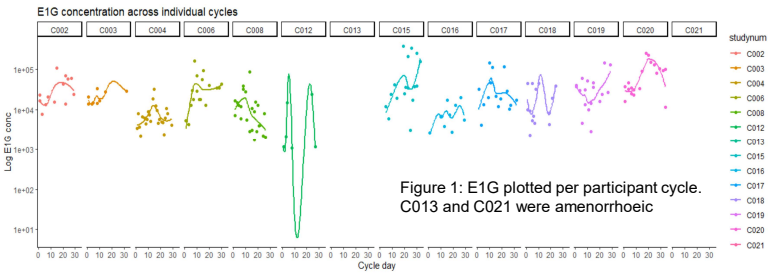
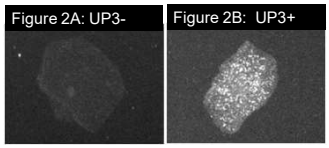


Figure 1: E1G plotted per participant cycle. C013 and C021 were amenorrhoeic

Immunofluorescence

86% of EPC were UP3+ cells (SD 74.5-96.8%)
Figure 2: A) UP3- non-urothelial epithelial cell, B) UP3+ urothelial epithelium



Linear regression

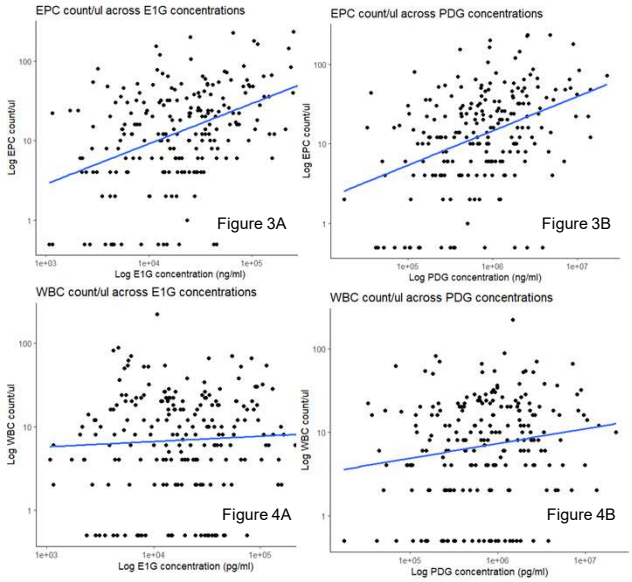
EPC count/µl had a significant linear relationship with hormones

- EPC vs E1G: adjusted R2 = .23, F-statistic = 21.6, p value < .00001 (Figure 3A)
- EPC vs PDG: adjusted R2 = .14, F-statistic 12.4, p value < .0008 (Figure 3B)

Contrastingly, WCC count/µl was not linearly associated with either hormone

- WBC vs E1G: adjusted R2 = -.017, F-statistic = 1.6, p value = 0.99 (Figure 4A)
- WBC vs PDG: adjusted R2 = -.017, F-statistic = .02, p value = .88 (Figure 4B)

RBC count/µl was also not linearly associated with either WBC or EPC count/µl (adjusted R2 = -.05, F-statistic = 0.2, p value = 0.84)



Conclusion

We have shown, for the first time, that epithelial cells found in full void urine samples do not indicate contamination. Instead, in healthy women, EPC appear to be shed in response to hormonal fluctuations. Conversely, WBC populations found in the urine appear to not have a relationship with urinary hormones but may fluctuate in response to transient sub-clinical infection. These data could be used to inform the design of novel and more fit-for-purpose diagnostic tests for urinary tract infection (UTI).

Dr Natasha Liou, MBBS, MSc (hon), MRCOG
Email: natasha.liou@gmail.com
Royal Free Campus

1. Foxman B, Brown P. Epidemiology of urinary tract infections: transmission and risk factors, incidence, and costs. Infect Dis Clin North Am. 2003 Jun;17(2):227-41. doi: 10.1016/s0891-5520(03)00005-9. PMID: 12848468.
2. Walter FG et a,. Squamous cells as predictors of bacterial contamination in urine samples. Ann Emerg Med. 1998 Apr;31(4):455-8. doi: 10.1016/s0196-0644(98)70253-7. PMID: 9546013.
3. Roos J et al. Monitoring the menstrual cycle: Comparison of urinary and serum reproductive hormones referenced to true ovulation. Eur J Contracept Reprod Health Care. 2015;20(6):438-50. doi: 10.3109/13625187.2015.1048331. Epub 2015 May 27. PMID: 26018113.