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LETTER TO THE EDITOR

# Differential profile analysis of urinary cytokines in patients with overactive bladder: reply

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Dear Editor,

We thank Dr. Cartwright and his colleagues [1] for their interest and comments on our paper [2].

When we designed our study, we strove hard to achieve a homogeneous group of women with overactive bladder. Our inclusion and exclusion criteria were strict. We also did not include men or children, or even post-menopausal women. In addition, we added sex-matched, age-matched controls. Our controls were two groups: positive control with documented urinary tract infection and negative control of the normal. We also measured all symptoms by overactive bladder symptom score. Finally, Dr. Abdel-Mageed's laboratory was blinded to the urine sample collected.

1. Our data were analyzed using Wilcoxon signed-rank test, a non-parametric statistical hypothesis or paired difference test, comparing two related samples or repeated measurements on a single sample to assess whether their population mean ranks differ. It is used as an alternative to Student's *t* test when the population cannot be assumed to be normally distributed or the data are on the ordinal scale. In our study, we used this test (at $p < 0.05$) to compare expression profiles of 120 human cytokines in urine samples of overactive bladder (OAB) or UTI relative to control subjects. Considering the sample size of 20 per group, this method would be more appropriate to use to test our hypothesis.
2. We clearly provided a thorough discussion on potential reasons for not detecting differences in the expression of the nerve growth factor (NGF) in urine samples of OAB patients compared to control subjects. Our NGF finding was supported by other studies cited in our paper. Many factors could influence the outcome of such studies, including patient populations, quality of urine samples, and time of urine collection and processing. Importantly, validation and characterization of affinity capture reagents (e.g., antibodies) might influence the outcome in proteomics research platforms adopted by various research laboratories in their respective field of study. This view was extensively discussed in a National Cancer Institute workshop [3]. Thus, it is apparent that the role of NGF in OAB is still a matter of conjecture.
3. Data presented in our Fig. 2 were not performed using analysis of variance (ANOVA). This was primarily attributed to the small sample size of our studies. It is rather a summary of selective and shared overexpressed cytokines based on Wilcoxon signed-rank test on urine samples of OAB or UTI relative to controls. We agree that such findings would be better analyzed using ANOVA and multiple comparison test in a large cohort of patients.

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