

## Peer Review File

Article information: <http://dx.doi.org/10.21037/tau-20-920>.

### Reviewers' comments

This is a timely article that addresses at length the potential association of *Ureaplasma Parvum* serovars with chronic micturition urethral pain in female patients having microscopic haematuria. It is interesting in that it has identified what appears to be a relationship between particular serovars of the pathogen and chronic micturition urethral pain.

I do think however that the paper would be strengthened if the concepts it is hoping to define are better characterized.

### Comment 1

Could the authors please clarify how they determined if a woman's infertility was "tubaric" or "uterine"? It is an interesting association they have identified however infertility is a complex issue for which many factors both biological and psychosexual are related. I think that while it is an interesting hypothesis that *Ureaplasma* serovars may be associated with infertility, the authors would benefit in better defining what they meant by infertility in the first instance. Following this, they could then define what they meant by "tubaric" or "uterine" infertility.

### Author's reply to comment 1

We truly appreciate to the reviewer for thoughtful and helpful comments. We totally agree with the reviewer's opinion about clarifying the types of infertility associated with *Ureaplasma parvum* (UP) infection.

Tubal infertility, which accounts for 25% - 35% of female infertility, is defined as tubal obstruction or occlusion (proximal, distal, unilateral or bilateral) inhibiting the movement of ovum into the uterus through the uterine tubes. As currently recommend by many studies regarding female infertility, hysterosalpingography was performed to all female patients visited to infertility clinic at our hospital. For the cases suspicious of tubal obstruction, diagnostic laparoscopy was additionally undertaken to make confirmative diagnosis as well as potential treatment, such as tuboplasty or pelvic adhesiolysis.

Uterine infertility, which is also called uterine factor infertility, may affect up to 1 in 500 reproductive age women and it can be sub-classified into two types, congenital uterine anomalies and acquired uterine factor infertility. For congenital anomalies, there are several etiologies including arcuate uterus, septate uterus, unicornuate/bicornuate uterus and mullerian agenesis. The diagnosis of uterine factor infertility was made by medical/menstruation review, transvaginal ultrasound (TVUS) and hysterosalpingography. Pelvic MRI was performed for the cases suspicious of adenomyosis co-presence. In the current study, 4 patients had congenital type uterine factor infertility among 73 infertile patients. However, all 8 infertile patients who were positive for urinary UP infection, had only acquired type uterine factor infertility but no congenital type anomalies. Among these 8 infertile patients with urinary UP infection, 6 patients had become infertile due to uterine synechiae whereas the other 2 patients were due to endometriosis. To clarify the definition of infertility types in the main text and table 1, tubal and uterine infertility have been renamed as tubal factor infertility and uterine factor infertility, respectively. Although our results suggested possible associations between tubal/uterine factor infertility with urinary UP infection, we did not explain the etiologies and diagnostic procedures of infertility in detail because this study is primarily focused on the urologic symptoms/signs including chronic micturition urethral pain (CMP) and microscopic hematuria (MH) as it was described in the discussion section of main text (page 15, line 345-347). We are hoping for the reviewer's and editor's generous understating on this matter.

### **Changes in the text (for comment 1)**

[Results, Page 11, line 245-248]

The **etiologies** of infertility for **the females**, confirmed with UTI by UP serotype-3 (8 patients), were either tubaric or uterine factors. In addition, the female infertile patients positive for urinary UP serotype-14 had their infertility resulted from only **tubaric factors**.

### **Comment 2**

**How was chronic micturition urethral pain defined? What were the specific symptoms that patients were asked for? What length of time did patients have the pain to have it defined as a chronic pain? Furthermore, among patients with chronic micturition urethral pain, were other aetiologies of pain excluded prior to them being included in the data set? How long were patient's pain free following treatment with Doxycycline?**

**If the authors wish to draw an association between chronic micturition pain and ureaplasma parvum serovars, they would benefit from better defining these points.**

**Author's reply to comment 2**

We truly appreciate to the reviewer's thoughtful comment as it helped us improving the quality of this article. We totally agree with the reviewer's opinion about clarifying the definition of chronic micturition urethral pain. During the visit to the urology outpatient clinic, all study subjects were asked whether they had experienced any discomfort perceived to be in the urethra during micturition. Moreover, chronic micturition urethral pain was defined when voiding urethral pain lasted more than 3-months. Due to the length limit of the article, we abbreviated the term chronic micturition urethral pain as CMP in the main article.

As it is mentioned in the method section (page 7, line 133-134), all study subjects were underwent abdomino-pelvis CT to exclude other possible causes of chronic micturition urethral pain. In addition, we believe any urogenital pathogens other than *Ureaplasma parvum* did not cause chronic urethral pain as all study cohort were screened with multiplex PCR according to the exclusion criteria, which has been explained in the method section of this article.

Regarding urethral pain free period after doxycycline treatment, all of the *Ureaplasma parvum* reinfection cases at 3-months follow-up maintained urethral pain free period at least 9 to 11 months after the initial doxycycline therapy. Nonetheless, these re-infected patients presented with voiding urethral pain at 3-months follow-up. The second doxycycline therapy removed urethral discomforts from the patients within 2 weeks. We hope this additional explanation will help answering the reviewer's inquiries.

**Changes in the text (for comment 2)**

[Methods, Page 7, line 130-134]

Regarding micturition urethral pain, all study subjects were inquired whether they had experienced any discomfort felt in urethra during urination, and voiding urethral discomfort lasted more than 3-months was defined as CMP in this study. Abdomino-pelvis computed tomography (CT) was also performed to all study subjects to detect the presence of urinary stones or other possible causes of CMP.

[Results, Page 12, line 271-275]

All of the reinfection cases accompanied micturition urethral pain at 3-months follow-up whereas the corresponding urethral discomfort reappeared 9- to 11-weeks after the initial antibiotics therapy. However, the patients, who were negative for UP reinfection at 3-month follow-up exam, maintained urethral pain-free-state.

### **Comment 3**

**Ureaplasma is currently arguably believed to be either a urological commensal or a sexually transmitted organism. Were patient's who had the symptoms described and in the data set ever screened for other sexually transmitted diseases? Were their partners ever screened to see if they also cultured the ureaplasma?**

### **Author's reply to comment 3**

We truly appreciate to the reviewer's thoughtful and helpful comment. We totally agree with the reviewer's opinion about other sexually transmitted organism.

We believe there was no other urogenital pathogen infection in the final study cohort as patients underwent multiplex-PCR with QIAamp DNA Mini Kit (Qiagen, Germany). This diagnostic procedure detects 12 different sexually transmitted infection (STI) pathogens including Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), Mycoplasma hominis (MHO), Trichomonas vaginalis (TV), Ureaplasma urealyticum (UU), Mycoplasma genitalium (MG), Herpes simplex virus (HSV)-1 and -2, Gardnerella vaginalis (GV), Treponema pallidum (TP), Candida albicans (CA) and UP. This STI related explanation has been presented in the method section, page 7, line 141-142 and line 165-173.

The male spouses of the patients were advised to undertake diagnostic test for Ureaplasma infection. However, we are afraid that we do not have complete medical information of those male spouses as this study focused on female study subjects. We hope this additional explanation will help answering the reviewer's inquiries.

### **Changes in the text (for comment 3)**

[Methods, Page 7, line 141-142]

Anyone tested positive for UTI or **vaginal infection** with pathogens other than UP according to multiplex PCR tests were also excluded from the analysis.

