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LETTER TO THE EDITOR

Male Infertility

A nonobstructive azoospermic patient with *Trichomonas vaginalis* infection in testes

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Dear Editor,

Trichomonas vaginalis (*T. vaginalis*), a flagellated protozoan parasite emerged as one of the most common nonviral sexually transmitted infections worldwide, often inhabits the vagina, urethra, prostate, and epididymis.[1] It has been estimated that there are more than 170 million new cases of *T. vaginalis* infections per year worldwide. However, current knowledge of *T. vaginalis* and trichomoniasis is based mainly on studies in female vaginal infections. The prevalence of trichomoniasis in males is far less well characterized than that in females, probably because the infection seems to be asymptomatic in most men and can be resolved after treatment with one dose of metronidazole.[1–3]

Among men, trichomoniasis has been considered as a cause of nongonoccocal urethritis (NGU) and as involvement in the impairment of male fertility.[1,3] *T. vaginalis* is found more often in infertile men than that in fertile individuals and its presence in semen results in significant decreased sperm parameter values, such as motility, normal morphology and viability.[4] *In vitro* studies have also shown that *T. vaginalis* and its secretory products reduce sperm motility and fertilizing capacity.[4,5] Although *T. vaginalis* has been identified in urethral discharge, urine, semen, and prostatic fluid, its infection may occur in other areas of the urogenital system. In rare cases reported, *T. vaginalis* infects the epididymis and prostate gland and occasionally, the testis.[4,6,7]

Herein, we report a novel case of nonobstructive azoospermia (NOA) with *T. vaginalis* infection in the testis. A 32-year-old male patient (1.76 m height and 90 kg weight), married for 10 years, presented with the complaint of infertility. General physical examination was normal and the ultrasound examination demonstrated normal epididymides, vasa deferentia, prostate, seminal vesicles, and ejaculatory duct. The testicular volume of each side was 6 ml, somewhat less than normal. Repeated semen analyses found no spermatozoa in the ejaculate, even after centrifugation. His endocrine profile listed in **Table 1** demonstrated that estrogen (E2) was a bit high while testosterone was low, which to a certain extent might be related to his obesity (body mass index [BMI] = 29.05). Genetic analysis showed normal 46, XY karyotype and no microdeletion of Yq azoospermia factor gene. No evidence of gross structural pathology was identified according to the formal urological evaluation and the diagnosis of NOA was given. At the same time, vaginal secretions from his wife were tested to be negative for trichomonas.

Therefore, we attempted retrieval of the patient's spermatozoa via surgical biopsy of the testes, which would be cryopreserved for further *in vitro* fertilization (IVF) treatment with his wife's oocytes. Wet preparations from fresh testicular biopsies from four locations in his right testis were examined for sperm under phase contrast microscopy and the pathological examination was performed at the same time. Sections of the testicular biopsies showed that very few germ cells, appearing to be spermatogonia and spermatocytes, were scattered in the seminiferous tubules, while spermatids were rarely detected (**Figure 1a**). The pathological diagnosis suggested a severe disruption of spermatogenesis. Meanwhile, wet preparations of testicular biopsies failed to demonstrate any sperm cells. However, some flagellated motile protozoa among numerous testicular and red blood cells were observed in one of the wet preparations (**Figure 1b** and **Supplementary Information**). In an attempt to identify the protozoa-like structures, Wright-Giemsa staining was made on the same day. On the basis of the morphological features of the cells, namely, an amoeboid shape, the presence of one elliptically shaped nucleus and poorly defined cytoplasm (**Figure 1c**), a provisional identification of *T. vaginalis* parasites was made.

Furthermore, laboratory PCR analysis was notable for the identification of this parasite. Briefly, genomic DNA was prepared from 1.5 ml of semen or 5 ml of urine using DNA extract kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. PCR was performed to check the expression of Tvk and BTUB, according to a procedure described previously.[8–10]

Table 1: Hormonal profile of the patient

Hormonal profile	*Value*	*Normal range*
Follicle-stimulating hormone (mIU ml^{-1})	12.2	1.5–12.5
Luteinizing hormone (mIU ml^{-1})	5.5	1.7–8.6
Prolactin (ng ml^{-1})	4.9	4.8–23.3
Estrogen 2 (pg ml^{-1})	53.7	7.6–42.6
Testosterone (ng ml^{-1})	1.8	2.8–8.0

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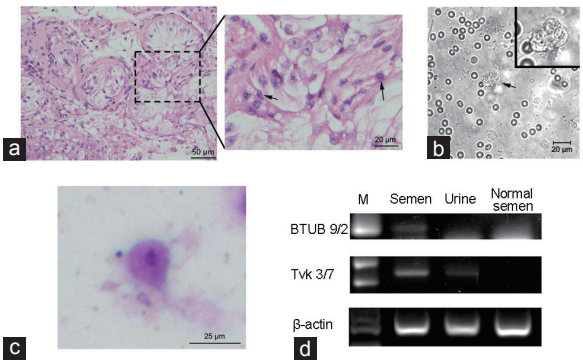

Figure 1: (**a**) HE staining of testicular biopsies, showed a severe disruption in spermatogenesis. Arrows indicate the germ cells. Scale bars = 50 μm (left) and 20 μm (right). (**b**) Wet preparation of testicular biopsies, showed a Trichomonas-like flagellate (arrow). Scale bar = 20 μm. (**c**) Wright-Giemsa staining of the wet preparation smear, arrow indicates *Trichomonas vaginalis*. Scale bar = 25 μm. (**d**) PCR analysis of *Trichomonas vaginalis* from the semen and urine. A normozoospermic semen (normal semen) sample was applied as negative control. M: 100 bp DNA ladder. HE: Hematoxylin-Eosin; PCR: polymerase chain reaction.

The results of PCR showed approximately 261 bp fragments representing Tvk 3/7, which has previously been shown to be the most sensitive conventional PCR test for *T. vaginalis*,[8] in the DNA extracts of both the semen and the urine. Likewise, results also showed an approximately 112 bp fragment representing BTUB 9/2, although a bit weak, in the DNA extract of the semen (**Figure 1d**). Therefore, both semen and urine from the patient were considered positive for *T. vaginalis*, and the NOA symptom may be caused by *T. vaginalis*-induced orchitis.

To our knowledge, this clinical case represents the first report of NOA related to *T. vaginalis* infection at the level of the testis. Combined with the existing reports,[3,4,6,7] it illustrates that spermatogenesis failure resulting from *T. vaginalis* infection in testis may be accompanied by low serum testosterone and atrophic testes, which may reflect the cytotoxicity of *T. vaginalis* in damaging germ cells and Leydig cells. Therefore, *T. vaginalis* infection in testis, although occasional, can seriously injure the niche essential for spermatogenesis. Meanwhile, male trichomoniasis is almost asymptomatic and few cases are diagnosed and treated. Hence, the infection persists, and males with a long-term trichomoniasis, such as 8 or 10 years, are more likely to suffer from NOA and become infertile. Although the role of trichomonas infection in pathogenesis of NOA and infertility is still unclear, this case also illustrates the importance of careful diagnosis and timely therapy for trichomoniasis.

On the other hand, it is noteworthy that the rare cases of *T. vaginalis* infection in the testis may imply a potential defect in the natural defense of the male urogenital tract. As is well known, innate host defense mechanisms in the male genital tract, such as cytokines, epithelial barrier, and epididymal macrophages, are critical for the defense against potential pathogens and provide an appropriate microenvironment for germ cell development and sperm maturation.[4] The presence of *T. vaginalis* in the epididymis, as well as in testis, suggests that the immune barrier is impaired while the protozoa travel through the winding genital tract. Thus, the association between chronic infection by *T. vaginalis*, inflammation, and a defect in defense of the male reproductive system should receive attention. Above all, it is important to improve the microenvironment of the urogenital tract in defending against pathogens during the therapy for trichomoniasis.

AUTHOR CONTRIBUTIONS

CX and ZL designed the experiment. YHG and YL performed the experimental work and participated in the pathological work. PL, ZJZ, and YH provided assistance in sample collection and treatment. GHF and YJX participated in the pathological work. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

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Supplementary information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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