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Parasites observed in urine sediments: A learning from incidental rare species

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ABSTRACT

Parasitic infection becomes an unsolved problem in using of automated instruments for urinalysis. Most common parasitic infections described in literature are Trichomonas vaginalis, Schistosoma hematobium and Microfilaria. Other rare organisms are Balantidium coli and Paramecium. The main purpose of this article is to raise the awareness in pathologists and clinicians on the importance of cytological evaluation of urine. This study was done to find the associations between the age and point prevalence of organisms in our zone in the last 6 years. Identification of parasites in centrifuged deposits of urine sediment is a relatively rare occurrence in clinical practice. A fairly wide morphological spectrum of parasites may be diagnosed through microscopic examination of centrifuged urine sediment. They may present with symptoms like pyuria, hematuria, diarrhoea, burning micturition, itching and morphological awareness helps in prompt and effective management in most cases.

1. Background

Urine is considered the “poor man’s renal biopsy," an ideal diagnostic specimen for its non-invasive and easy method of collection. Urinalysis encompasses a wide range of tests, which include a variety of physical analysis, chemical tests, urine microscopy, bacterial cultures, and molecular tests.[1] Importantly, urine tests can diagnose patients with parasitic infections.

In this machine-based era, these organisms are easily missed, and though doing things manually becomes cumbersome, microscopic examination of urinary sediments is very important for the diagnosis of these organisms. Various motile organisms that can be seen in urine are Trichomonas, Micofilaria, and Schistosoma hematobium, and other rare organisms are B. coli and Paramecium.

In the present study, we incidentally found the rarest of organisms on urine examinations. Balantidium coli (B.coli) is the only parasitic ciliate of man. It encompasses worldwide distribution. The prevalence of B. coli in human hosts may vary from 0.02% to 1%.[2–4] In humans, the infection is found mostly in areas of tropical and subtropical regions and developing countries, with poor environmental and sanitary conditions.[2] Higher prevalence of B.coli was reported in Latin America, Philippines, Papua New Guinea, and the Middle East, with about 29% reports among pig farmers.[2,5–9] Currently, there are about 11 documented cases of urinary balantidiasis reported globally. Single cases were reported from Iran,[10] Italy,[11] Slovenia[12] and Thailand[13], while 7 cases were reported from India.[14–19]

The low frequency of B.coli may not represent the real occurrence and prevalence of the parasite as it usually remains asymptomatic and under reported. The main mode of transmission for B. coli is the feco-oral route, usually occurring via ingestion of food or water contaminated

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with the cyst stage.[10] The infection fundamentally affects the colon and causes variable clinical pictures, from asymptomatic to serious dysenteric forms. The greenish-yellow trophozoites can grow to be 120 × 150 μm long and can attack the intestinal epithelium, causing ulcers and bloody diarrhoea similar to amebic dysentery.[20]

We also found dual infections of B. coli and Paramecium. Paramecium, a genus of microscopic, single-celled, and free-living protozoans, is non-pathogenic to humans. It is found in fresh water, including ponds, rivers, lakes, streams, and pools. Microscopically, it is slipper-, cigar-, or spindle-shaped. The entire body surface is covered by numerous, tiny, hair-like protoplasmic processes called cilia. Endoplasm is semi-fluid and less granular.[21]

Filariasis is a widespread public health problem seen commonly in tropical countries. W. Bancrofti accounts for the majority of the filarial infection in India, accounting for approximately 95% of cases. However, Microfilariae is very rare to be detected in routine urine. Infection by this sheathed species is commonly seen in India, China, Indonesia, and the Eastern Pacific.[22] The common presentations include microfilaremia, lymphedema, hydrocele, acute adenolymphangitis (ADL), chronic lymphatic disease, and less common presentations like chyluria and tropical eosinophilia. Achylous hematuria is a very rare presentation. However, almost all filariasis patients have microscopic hematuria and/or proteinuria. Shedding of microfilaria in urine is possibly determined by local factors like inflammation, trauma, or stasis, which mainly affect the lymphatics and small vessels, causing either lymphatic blockage or damage to the vessel wall. To the best of our knowledge, only one case of W. Bancrofti in achylous urine has been reported.[23]

Trichomoniasis, which is caused by Trichomonas vaginalis (T. vaginalis), affects 30.1 million people in the WHO Western Pacific Region and 187 million people globally, making it the most prevalent non-viral sexually transmitted infection.[24] Trichomoniasis is the most common non-virally transmitted infection worldwide.[25] T. vaginalis has been reported to cause several human infections, typically of the urogenital organs, such as vaginitis, urethritis, and prostatitis.[26] The symptoms of T. vaginalis infection vary; up to 50% of female patients exhibit no symptoms.[26] T. vaginalis infection causes symptoms similar to a urinary tract infection, such as dysuria and urinary frequency and urgency.[27] Its diagnosis traditionally performed by microscopic examination or culture of vaginal fluid.

The current medical literature has many isolated case reports for urinary parasitic infections; however, the studies on these in the Indian population are limited. The current study sought to determine the prevalence of rare parasites in routine urine examinations as well as their clinical manifestations.

2. Materials and Methods

A Descriptive study including both prospective and retrospective studies, compiling the types of urinary parasitic infections that had been reported in routine urine analysis over a period of 6 years (January 2017 to January 2023) at Government Doon Medical College, Dehradun, Uttarakhand, India. A midstream or catheterized urine samples collected in a sterile container (30ml) were received in the clinical pathology laboratory. Urine was processed and examined within 1 hour of collection. The sample was subjected to an automated urine analyzer for routine physical, chemical, and microscopic examination. The gross examination was rechecked by the doctor on duty. The parameters analysed by the urine analyzer were blood, bilirubin, urobilinogen, ketones, protein, nitrite, glucose, pH, specific gravity, and leucocytes. As far as the quality of microscopic findings is concerned, the urine analyzers fail to detect parasitic organisms and also have a tendency to give false microscopic results. Therefore, all urine samples are examined as wet mounts under the microscope. In order to prepare sediment, 10–15 ml of urine was centrifuged at 2500 rpm for 10 min. The sediment are then put as a drop on a slide and covered with a cover slip to prepare a wet mount. The examination was carried out by lowering the condenser and observing first under the scanner, followed by low-power and high-power fields.

3. Observations

The following results were observed and calculated in present study are as follows [Table 1]

Fig. 1: Trophozoite stage of Balantidium coli, having a mouth (blue arrow) along with its cyst (yellow arrow) found in an elderly female urine[Wet mount, high power].

Table 1: Clinical details and urine routine & microscopic findings of case reported in present study.

Case	Age/Sex	Clinical presentation	Physical examination	Chemical examination	Diagnostic finding	Microscopic examination	Other laboratory findings
1.	79 yr/ F	Fever, Loose stools, weakness in legs	Yellow colored turbid urine pH = 8.0 Specific gravity = 1.015	Albumin + Sugar – nil Blood- nil Biliirubin –nil Urobilinogen – nil Ketone bodies - nil	Few large cigar to ovoid shaped ciliated parasites (approximately $200 \times 50\mu$) were seen, swim rapidly across the slide **(Fig. 1).** The organism had a mouth that was located in the tapering anterior end (cystosome) and a rounded posterior end (cytopyge). Several food vacuoles, macronucleus and few ingested red blood cells were present within cytoplasm. The body was covered with pellicle with longitudinal striation, along with short delicate cilia all around, of uniform length. The cilia lining the mouth part appeared to be longer than others. The morphology and swimming pattern was characteristic of B. Coli. Few oval to round cysts are also noted, size 60-80 μ with a bean shaped macronucleolus ,s/o B.Coli Cysts [Figure 1] Also, fast moving conoid to ovoid,cigar shaped and slender microraganism found ,size 50-60 μm. A Tumbling movement also seen revealing its groove just in between the body .morphologically s/o Paramecium. Coinfection of B. Coli & Paramecium.	Epithelial cells= 2-4/hpf Pus cells= 6-8 /hpf RBC= nil Crystals – Cholesterol Casts – Broad Bacteria – present	CBC= neutrophilia ESR= 25 mm/1^{st} hr B.Urea= 98 mg/dl S. Ceatinine =7 mg/dl S. Vit D = 125 pg/ml LFT- within normal limits MP, NS1Ag & Chikangunia –Negative Typhidot- Positive

Continued on next page

Table 1 continued

2.	78yr/F	Oligouria, comatosed patient	Yellow colored turbid urine pH = 5.0 Specific gravity = 1.010	Albumin +++ Sugar – nil Blood + Biliirubin –nil Urobilinogen – nil Ketone bodies - nil	Few oval to round cysts are also noted, size 50-60 μ with a bean shaped macronucleolus ,s/o B.Coli Cysts. However, fair number of oval fat bodies are noted in the background , only few cysts were a challenge to diagnose microscopically . But, stool examination also showed B.Coli Cysts . [Figure 2]	Epithelial cells= 8-10/hpf Pus cells= 6-8 /hpf RBC= 1-2 Crystals – nil Casts – nil Others – lipid droplets	Hb= 9.4 g/dl SGOT = 58 IU/L Total proteins =6.4 gm/dl A/G ratio= 1.1 B.Urea= 81 mg/dl S.Creatinine=3.1 mg/dl BUN=37.9 mg/dl S.uric acid=7.4 mg/dl Sodium=134 MMOL/l Pottassium=6.2 mmol/L Trop T HS= 521.3 pg/ml CPK-MB= 10.6 microgram/l Procalcitotin = 0.09 ng/ml Amylase= 229 U/L S.Lipase= 74 U/L ADA=2.0U/L Pleural fluid-polymorphs
3.	15 yr/F	Burning& itching while micturition	Yellow colored turbid urine pH = 8.2 Specific gravity = 1.012 .	Albumin + Sugar + Blood-nil Biliirubin –nil Urobilinogen – nil Ketone bodies - nil	Fast moving conoid to ovoid,cigar shaped and slender microraganism found ,size 50-60 μm. A Tumbling movement also seen revealing its groove just in between the body .morphologically s/o Paramecium [Figure 3]	Epithelial cells= 2-3 /hpf Pus cells= 4-6/hpf RBC= 1-2 Crystals – Calcium Oxalate ++ Casts – nil Bacteria – present Fungal pseudohypae seen ,morphologically s/o Candida spp	CBC= Hb=9.6 g/dl TLC=14000 /cumm, DLC= 88 % neutrophils ESR= 32 mm/1st hr

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Table 1 continued

4.	41 yr/M	Fever,swelling in legs and groin	Blood tinged dark yellow colored turbid urine pH = 7.4 Specific gravity = 1.022	Albumin + Sugar – nil Blood- present Biliirubin –nil Urobilinogen – nil Ketone bodies - nil	A single elongated motile organism seen ,morphologically s/o Microfilaria. For confirmation, a cytology smear is prepared from urine sediment, show a sheathed organism with granular body, however granules are absent in tail. A diagnosis of Microfilaria had been made.	Epithelial cells= 1-2/hpf Pus cells=30-40 /hpf RBC= Full field Crystals –nil Casts – nil Bacteria – present	CBC= Hb-12.6 g/dl TLC =16,800 /cumm leucocytosis with shift to left Platelet-90,000/cumm ESR= 14 mm/1st hr
5.	29yr/F	Burning& itching while micturition, vaginal discharge x 5 months	Straw colored turbid urine pH = 6.8 Specific gravity = 1.011	Albumin- Trace Sugar – nil Blood- nil Biliirubin –nil Urobilinogen – nil Ketone bodies - nil	Fair number of oval to kite shaped highly motile organisms seen with flagella at the end ,skipping the microscopy flield morphologically s/o Trichomonas vaginalis . Also , fair number of transitional cells are also noted in sheets and groups.	Epithelial cells= 10-12 /hpf Pus cells= 16-20/hpf RBC= nil Crystals – uric acid Casts – nil Bacteria – present	CBC= Hb-8.6 g/dl TLC =13,200 /cumm, Neutrophilia S.Uric acid= 11.6 mg/dl
6.	32 yr/**M**	Burning while micturition	yellow colored turbid urine pH = 8.0 Specific gravity = 1.010	Albumin +++ Sugar – nil Blood- nil Biliirubin + Urobilinogen – nil Ketone bodies - nil	Fair number of Trichomonas vaginalis seen.	Epithelial cells= 6-8 /hpf Pus cells= 10-12/hpf RBC= nil Crystals – triple phosphate Casts – granular Budding yeasts seen ,morphologically s/o Candida spp	Not available
7.	28yr/F	Primary Infertility,fevver, burning mictiturition	straw colored slightly turbid urine pH = 8.6 Specific gravity = 1.021	Albumin-nil Sugar – nil Blood- nil Biliirubin -nil Urobilinogen – nil Ketone bodies - nil	Fair number of Trichomonas vaginalis seen	Epithelial cells= 4-6/hpf Pus cells= 12-14 /hpf RBC= nil Crystals – nil Casts – nil Bacteria – present	CBC= Hb=9.8 g/dl TLC=14,300/cumm, DLC= 78 % neutrophils ESR= 18 mm/1st hr TSH= 0.587 uIU/ml

1. A total of 8500 urine samples were examined, and 7 cases (0.08%) of parasitic infection were discovered on urine microscopy.
2. The infected patients presented in wider age, ranged from 15 to 79 years, with an average age of 43.1 years. The maximum number of patients was in the 7th decade, followed by the 3rd decade.
3. Female (71 4% predominance was seen in a ratio of M:F = 1:2 5
4. In our present study, we found two cases of paramecium. The first case involved an elderly female who had a dual infection of B. coli and paramecium. A second case was seen in a 15-year-old female, which was found to be associated with numerous sheets of transitional cells and with candidal pseudohyphae. We could not do further diagnostic investigations in reference to sheets of transitional cells, as the patient did not follow up.
5. Two cases of Balantidium coli cysts, one without active B.coli trophozoite are reported in females, both were in similar geriatric age group. The intriguing thing was that both females had deranged renal function tests with raised serum creatinine, serum urea, and albumin.
6. We found one case of microfilaria incidentally in achylous turbid urine with a high TLC count and shift to left on GBP. It is found to be associated with pyuria and massive hematuria. Detection of microfilaria in voided urine sediment, especially in achylous hematuria specimens, is extremely rare.
7. The most common parasite in our study was T. vaginalis. Three cases of T. vaginalis were seen, predominantly in females; with M:F ratio of 1:3. One amongst them, have a history primary infertility from 3 years. The present study revealed an adult male in his 30s showed this intriguing parasite in his urine. The characteristic morphology and "wobbling" and "rotatory" movements of the organism aid in specific diagnosis.
8. The pH of the urine varied from acidic to alkaline, i.e. 5 to 8.6, with an average of 7.4. A female patient with dual infection of B. coli cysts with Paramecium had the highest pH of 8.6, while a 79-year-old female with B. coli in urine had the lowest pH of 5.
9. The specific gravity ranged from 1.010 to 1.022, with an average of 1.014. However, static specific gravity was noted with B. coli cysts.
10. Urine albumin is found to be raised in 90% cases (n=6/7), varies from trace to 3+. One case of T.vaginalis showed absent urine albumin.
11. Glycosuria noted in single case (n=1/7 associated with paramecium
12. Bilirubinuria noted in single case (n=1/7 associated with T vaginalis.

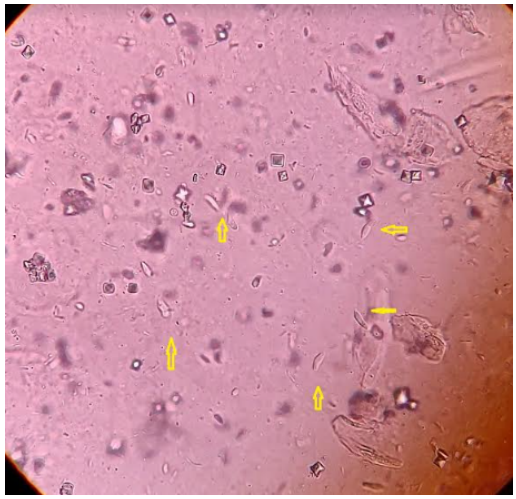

Fig. 2: Fair number of Paramecium (yellow arrow) and calcium oxalate crystals noted in routine urine [Wet mount, high power].

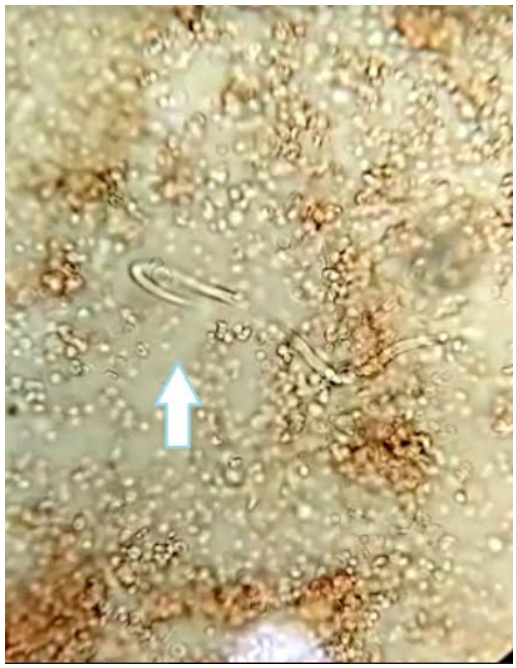

Fig. 3: Microfilaria (white arrow) associated with marked hematuria [Wet mount, high power].

4. Discussion

Urinary parasite detection is a relatively uncommon and incidental finding in routine urine examination. According to the literature, common urinary parasitic infections include Trichomonas, Schistosoma hematobium, and Microfilaria.[2] B. coli and Paramecium are two other uncommon organisms found in urine. There are only individual case reports on these parasites in the literature, and only one single study has been done on these urinary parasites collectively. We discovered very rare incidental

findings and interesting cases in urine examination in our study.

The majority of the patients in our study had urinary complaints, including burning and itching, while micturition (3/7), fever (3/7), vaginal discharge (1/7), primary infertility (1/7), loose stools (1/7), and oliguria (1%) were some of the additional complaints. Out of these patients, many had come for a routine check-up.

Urinary balantidiasis is rare. The first reported case in 2007 was a 60-year-old Indian man who complained of fever, lower abdominal pain, dysuria, and urinary frequency.[14] He had no underlying disease and no history of contact with pigs.[16] In other case reports, all patients had an underlying immune-compromising disease such as diabetes mellitus, steroid-treated chronic obstructive pulmonary disease, or chronic kidney diseasem[14,16–19] where the urinary balantidiasis was accidentally found during the urinalysis. Similarly, in our cases in which B.coli was found, both the patients were elderly female, immune-compromised, as they had deranged renal function; both experienced fever, leg pain, weakness, and oliguria and one of them was in a comatose state. Balantidiasis is usually an asymptomatic infestation of the colon, but symptoms may manifest in patients with low immunity, and such patients may also experience systemic involvement.[2,28]

Bandyopadhyay A et al.[16] presented a case that was similar to our case finding, in which an elderly 72-year-old female patient presented with symptoms of mild fever, dysuria, increased frequency of micturition, and pelvic pain for 7 days. Urine microscopy showed dual infection of B. Coli and T. Vaginalis. In the present study, we also documented concomitant dual infection of paramecium and B.coli in an elderly female approaching her 80s.

There has only been one case report on paramecium in archives to date; Singh S et al.[29] reported a case in a 75-year-old male patient operated on for carcinoma of the larynx 5 years back and was admitted in a comatose stage with an inability to pass urine for the past 2 days.

Wuchereria bancrofti is responsible for the vast majority of filarial infections in India, accounting for approximately 95% of all cases.[23] Infection by this sheathed species is commonly seen in India, China, Indonesia, and the Eastern Pacific.[22] Webber and Eveland were the first to report microfilaria in both voided as well as catheterized urine samples from a young male patient with intermittent painless hematuria.[30] Filariasis has been reported in cytologic smears from various organs and sites, like male genital organs, thyroid, breast, lymph nodes, liver, soft tissue swellings, bone marrow, cervical smears, body fluids, etc.[30–36]

In the present case, microfilaria was incidentally detected in a voided centrifuged-day urine sample from a young male patient who presented with complaints of fever and swelling in one leg and groin region. However, grossly, the urine was blood-tinged, yellow in color and, turbid. There was no history of chyluria. The peripheral smear examination did not reveal any parasites. Also, we performed FNAC on the swelling in the groin region of the same patient and stained slides revealed microfilariae. This is the second case of achylous urine reported. The explanation of non-chyluric turbid urine may be that significant lymphatic obstruction may not have taken place; therefore, the patient did not present with chyluria. Similar to our case report, Ahuja et al.[23] reported a case of microfilaria in routine urine in a 25-year-old male patient who presented with intermittent painless achylous hematuria.

Detection of parasite DNA by polymerase chain reaction (PCR) is now considered the most sensitive technique for a definite diagnosis of parasitic infection. Even after many decades since it was first used for W. bancrofti infection, diethylcarbamazine (DEC, 6 mg/kg daily for 12 days) remains the treatment of choice for the individual with active infection. The medication is both macro- and microfilaricidal.[23]

T. vaginalis resides in the vagina and colonises the cervix; in men, it can be found in the urogenital tract and the prostate. T. vaginalis in males often goes undetected, and thus the incidence is difficult to determine. However, it has been reported that in men with infected female partners, the incidence can range from 15 to 73%.[14–17] Additionally, men can clear infections 3–12 times faster than women, whether the infection is treated or not.[18,19] Regardless, it is thought that a higher proportion of men than women have asymptomatic infections.[16,20–22] The characteristic morphology and "wobbling" and "rotatory" movements of the organism aid in specific diagnosis.

In the present study, T. vaginalis was seen in 3 cases, of which one was male and presented with burning micturition. A routine urine examination was also performed on a 29-year-old female, whose urine was turbid grossly and microscopically wet mount showed numerous sheets of transitional cells in urine, indicating that a diagnostic workup should be performed in such patients to determine the cause if there is any underlying malignancy of the urinary tract, but we were unable to trace this patient due to a lack of follow-up.

T.vaginalis is a cardinal parasite of the male urogenital tract and is an important sexually transmitted infection. Thus, the presence of this flagellate parasite essentially calls for treatment of both partners. Examination of urine within 1 hour of collection ensures the highest possibility of detecting these parasites.

Similar to our case, Montalbo R. et al.[37] did a prospective case study of 83 patients who had urine cytology that was suspicious of urothelial carcinoma with a negative cystoscopy and underwent a second cystoscopy and urine evaluation by cytology. The presence of tumour was identified in 41% of patients; of these, 82% had tumours

identified at their second evaluation (76% were high-grade [HG] tumors), and 18% had tumours identified at a later follow-up (50% were HG tumors).[37]

5. Conclusion

To summarize, routine urine examination is an important diagnostic examination of various microorganisms that machines frequently miss. However, the increased frequency of dysuria, pyuria, and hematuria observed in this study indicates a high likelihood of parasites being found in such patients' urine. A high index of suspicion and a meticulous search for the parasite in centrifuged urine sediments are thus indicated. In addition, identification of morphology with associated motility in vital preparation is an economical and convenient tool in clinical pathology practice.

6. Source of Funding

None.

7. Conflicts of Interest

There are no conflicts of interest.

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