Spectrum of parasitic infections in centrifuged urine sediments from a newly developed tertiary care centre in Central India

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Abstract Detection of urinary parasites is relatively rare and incidental finding in routine urine examination. Common urinary parasitic infections as described in literature include *Trichomonas*, *Schistosoma hematobium* and *Microfilaria*. *Trichomonas vaginalis* is known to cause vaginitis and urethritis, and may be found in urine sediments. In this study, the spectrum of urinary parasitic infections that had been reported in the last one and a half year was evaluated, and point prevalence in this zone was estimated. Microbiologist opinion had been taken in the difficult cases. Out of the total centrifuged urine sediments examined, urinary parasitic infection was found in 33 cases. The calculated point prevalence is 0.39%. Most common parasitic infection reported was flagellates (27 cases: 25 *T. vaginalis*, 2 commensal flagellate closest to *Chylomastix*), followed by three cases showing eggs of *Enterobius vermicularis*, one case showing larvae of *Strongyloides stercoralis* and two cases of ciliate protozoa. One of the ciliate protozoa was *Balantidium coli* and the other one was *Balantidium* like ciliate morphologically

closest to *Chilodonella* spp. Pyuria was found in 22 out of the 33 cases and hematuria in 17 out of 33 cases. A fairly wide morphological spectrum of parasites may be diagnosed through microscopic examination of centrifuged urine sediment. They may cause pyuria and haematuria, and morphological awareness helps in prompt and effective management in most cases.

Keywords *Balantidium coli* · *Enterobius vermicularis* · Protozoa · *Strongyloides stercoralis* · *Trichomonas vaginalis* · Urine sediment

Introduction

Identification of parasites in centrifuged deposits of urine sediment is a relatively rare occurrence in clinical practice. Parasites and parasitic ova may be seen in urinary sediments as a result of fecal or vaginal contamination (Mc Pherson et al. 2011). A common urinary parasite described in one study from Nigeria is *Schistosoma haematobium* (Morenikeji et al. 2009). *Trichomonas vaginalis* causing vaginitis and urethritis, may be found in urine sediments. Amoebae may rarely be seen in the urine by fecal contamination. The current medical literature has many isolated case reports for urinary parasitic infections; however the studies on these from the Indian population are limited. The present study aimed at estimating the point prevalence and analyzing the spectrum of parasitic infections in urine centrifuged deposits.

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Materials and methods

This was a retrospective study compiling the types of urinary parasitic infections that had been reported in the last one and half year. Most patients had submitted urine sample as a part of routine investigation or because of some urinary symptoms. Urine sample collected in a sterile container (30 ml) was processed and examined within 1 h of collection. The routine processing of urine involved pouring 10–15 ml of urine into a graduated disposable centrifuge tube. After carrying out the initial physical examination the chemical examination was performed. The chemical examination was done by commercially available strips. The parameters analyzed were blood, bilirubin, urobilinogen, ketones, protein, nitrite, glucose, pH, specific gravity, and leucocytes. Processing was done by centrifuging at 2500 rpm for 10 min and wet unstained sediments obtained were examined. The examination was carried out by lowering the condenser, observing first under scanner and low power field and then under high power field. The opinion of the microbiologist was also taken in difficult cases. The cases in which the wet preparation showed the parasite, Papanicolaou and Wright Giemsa stained smears were also prepared. Correlation with cervical smears and routine stool examination had been done wherever available. Urine examination was carried out on two occasions in the cases with ciliate protozoa infection to rule out any incidental fresh water ciliate contamination.

Results and observations

Out of the total of 8444 routine urine samples examined, urinary parasitic infection was found in 33 cases. All the patients had presented to the outpatient departments (OPDs). Out of these 33 patients; 14 (42%) patients had registered for gynecology and obstetrics OPD, 12 (36%) had registered for the medicine OPD, 4 (12%) had registered for the pediatrics OPD, and one each for the dermatology, orthopedics and neurology OPDs. The calculated point prevalence is 0.39% (confidence interval 0.27–0.54). The patients' age ranged from 6 to 69 years with an average age of 34.8 years. The maximum number of patients was in the 3rd decade followed by 2nd decade. Twenty-seven patients were females (82%) and 6 were males (18%) (Fig. 1).

The pH of the urine varied from 5 to 7.5 with an average of 6.2. The maximum pH of 7.5 was seen in a male patient showing *Balantidium coli* (*B. coli*) and the minimum pH was seen in a 40 year female with *T. vaginalis*. The specific gravity ranged from 1.005 to 1.030 with an average of 1.017. Glucosuria and proteinuria were seen in one case

Fig. 1 Histogram depicting age and gender distribution

each, with trichomonad infection. Two cases showed nitrite positivity on multistix examination, implying bacterial coinfection in addition to trichomonads. Crystalluria was seen in six cases, five cases showing calcium oxalate crystals and one case mixed triple phosphate and calcium oxalate crystals. One of the cases showing numerous round structures with peripheral lamellations was mistakenly diagnosed as eggs of *Hymenolepis nana* (*H. nana*). These were actually starch granules, and the stool examination did not reveal any parasite. This case was not included; however shall be discussed as a potential mimic.

Protozoan infections were seen in 29 cases and helminthic infection was seen in four cases. Most common parasitic infection was flagellates (27 cases; 25 *T. vaginalis*, 2 commensal flagellate closest to *Chylomastix*). One case of ciliate *B. coli* and another case showing dual infection with *Balantidium* like ciliate morphologically resembling *Chilodonella* and *Giardia* were observed. Three cases showed ova of *Enterobius vermicularis*. One case showed larvae of *Strongyloides stercoralis*. Pyuria was found in 22 and hematuria in 17 of these 33 cases. Amongst males the parasites observed were three cases of *T. vaginalis*, one case of *B. coli*, and two cases of commensal flagellate closest to *Chylomastix*.

Protozoan infections

The trichomonads were the most common protozoan parasite to be found in the deposits. The size of the parasite was somewhat bigger than the WBCs and smaller than epithelial cells (around 20 μ) (Fig. 2a, b). The number of trichomonads varied from case to case, ranging from occasional to numerous parasitic profiles. Flagella were better appreciated once the motility slowed down.

Ciliates were seen in the urine sediments of a young psychotic male and a geriatric female. This young male in his early 20 s presented with burning micturition and increased urinary frequency. Urine examination revealed

Fig. 2 **a** Sediments from centrifuged urine shows numerous pear shaped organisms along with many pus cells (Wet mount; 40 × objective). **b** The stained sediments show characteristic morphology of *Trichomonas vaginalis* (PAP; 40 ×)

pH of 7.5, specific gravity of 1.005, mild hematuria and pyuria (20–22 WBCs/hpf). The microscopic examination confirmed the pyuria and hematuria and showed trophozoites of organisms resembling *B. coli*. These organisms had an oval shape, size around 60 μ , had a mouth located in tapering anterior end (cytostome) and a rounded posterior end (cytopyge). The organism was briskly motile, often displaying rotator motion. One to two food vacuoles and a macronucleus were seen. The body was covered with short delicate cilia all around and they were of uniform length; the cilia lining the mouth appeared a little longer. The morphology and motility pattern was characteristic of *B. coli*. The patient's urine culture was sterile and HIV status was non-reactive. He mentioned history of recent bowel irregularity; however stool examination did not show any parasite. He was given oral metronidazole for 7 days and repeat urine examination did not reveal any parasite, and there was also no pyuria or hematuria. Another ciliate was seen in a 69-year-old woman presented with urinary symptoms. These organisms were 20–30 μ in length and 20 μ in diameter, and were smaller than the surrounding epithelial cells (Fig. 3a, b). They were asymmetrical oval (vase) shaped covered with cilia, with convexity on one side and mild concavity on the other upper aspect. The cilia appeared to be longer and better appreciable on one aspect (ESM_1.mpg), and were lining a vestibulum leading to a cytostome. Ovoid macronucleus and a minor nucleus were seen. Occasional cyst was also seen on the stained smears. Repeated urine examination confirmed the presence of ciliate and showed numerous flagellates with characteristic “falling leaf” motility. The stained smears showed binucleate flagellates which were 12–15 μ in length and 8–10 μ in diameter. Repeat stool examination carried on 2 consecutive days was negative for any parasite. Based on their motility, cytomorphological findings and review of

available literature, a diagnosis of co-infection with *Balantidium* like ciliate resembling *Chilodonella* spp. (Khurana 2018, accepted, Journal of Cytology) and flagellate *Giardia* spp was considered. The patient was treated with oral metronidazole 400 mg thrice daily for 7 days, and subsequent urine examination was free of any infection.

Helminthic infections

The eggs of *E. vermicularis* were seen in urine sediments of three school girls, one was 6 year old and the other two were 7 years. Two of them presented with urinary complaints and one had joint pains in addition to urinary complaints. Peripheral blood eosinophilia was observed in one of the cases. Urine examination showed pyuria in one, hematuria in the second and hematuria as well as pyuria in the third patient. These were characteristic ova with plano-convex morphology, varying in size from 70 to 90 μ making them visible under the scanner and low power lens (Fig. 4a, b). One to two eggs were seen in the centrifuged deposits. On wet mount preparation larva inside the egg showed a light yellowish-green hue and appeared as a crescent showing a gradual movement on intricate observation.

A 40 year-old-female presented to the gynecology OPD with complaints of pain in lower abdomen, increased frequency of urine. The patient was not diabetic and her HIV status was not known. Per speculum examination showed candidiasis and bilateral fornices were tender on per-vaginal examination. No hematuria or pyuria was seen. Urine sediments showed larvae of *Strongyloides*. The organisms were around 90–110 μ in length and 10 μ in width, with a ventral sucker; no ova were observed even on extensive search (Fig. 5; ESM_2.mpg). No lateral alae and

Fig. 3 **a** Urine sediment showing trophozoites of *Balantidium* like ciliate parasite closest to *Chilodonella* (wet mount; 40 × objective). **b** MGG stained sediment of the same case (MGG; 40 ×)

Fig. 4 **a** Urine sediment showing a planoconvex egg of *E. vermicularis* with a coiled up larva, and few RBCs in the background (wet mount; 10 ×). **b** PAP stained sediment of the same case (PAP; 10 ×)

Fig. 5 Urine sediment showing larvae of *Strongyloides stercoralis* (wet mount; 10 ×)

absence of ova excluded the possibility of enterobiasis. The patient was advised stool examination and HIV serology but she did not give consent for the tests. She was advised albendazole, Fluconazole, and was asked for a follow up visit the next week; however the patient did not come for any follow up.

Cellulose tape/stool examination was advised in all the cases with urinary parasites other than *T. vaginalis*, and was submitted in four out of the eight cases. One case confirmed the presence of eggs of *E. vermicularis*. In another case showing eggs of *E. vermicularis* in urine, the patient submitted the stool examination post treatment. In the case showing dual infection with *Balantidium* like ciliate and *Giardia*, stool examination revealed only the flagellate. One of the boys showing presence of *Chylomastix* in urinary sediments, showed presence of cysts of *E. histolytica* along with this commensal flagellate in the stool examination. One case showing starch granules on urine examination, mimicking eggs of *H. nana* was erroneously

Fig. 6 Starch granules can mimic the eggs of parasites like *Hymenolepis nana*, a potential mimic (wet mount; 10 ×)

diagnosed (Fig. 6). Ova were not seen on stool examination in this patient.

Six out of the 33 (18%) patients showed co-infection in the urinary sediments. In two of the cases trichomonads infection was seen together with pathogenic bacteria as nitrite was positive on chemical examination and cultures were also positive. In one case trichomonad infection was seen together with candidiasis. In one case co-infection of *Balantidium* like ciliate *Chilodonella* and *Giardia* were seen. In the young boy with *Chylomastix* flagellates in urine, the stool examination also showed coinfection with *Entamoeba Histolytica*. In the female patient having *Strongyloides infestation*, the urine examination also showed yeast forms of *Candida*. Correlation with cervical smears was available in nine cases. In eight cases Pap smear examination showed reactive cellular changes associated with inflammation and presence of *T. vaginalis*; however the organism was missed in one case.

Discussion

Three common parasites that can be found in urine are *Trichomonas*, *Schistosoma hematobium* and microfilaria (Cheesebrough 2009; Mc Pherson et al. 2011). *Schistosoma* infection (Bilharziasis) is not common in India (Kali 2015) and filarial infection is also uncommon in central India. In the present study the females outnumber the males. Fecal contamination of urine sample in females is anatomically much more probable than males and vagina is a favorable habitat for *T. vaginalis* which constitutes the most common parasite. The maximum number of patients was in the 3rd decade followed by 2nd decade. *T. vaginalis* was the most common parasite seen, possibly because it is a sexually transmitted infection, and most patients belonged to the sexually active age groups. Enterobiasis was seen in young girls as this is the age children have intestinal helminthic

infestations and fecovaginal/urinary tract contamination is anatomically more probable in female gender. Vaginal parasitosis has been reported with *T. vaginalis*, *E. vermicularis*, *S. stercoralis*, eggs of *Ascaris lumbricoides* and microfilariae (Mali and Joshi 1987). Amongst males the parasitic infections were found in three cases of *T. vaginalis*, one case of *B. coli*, and two cases of commensal flagellate closest to *Chylomastix*.

Trichomonas vaginalis causing vaginitis and urethritis, is a common vaginal parasite seen in urine examination (Blake et al. 1999). Diagnosis of this infection is traditionally performed by microscopic examination or culture of vaginal fluid. Blake et al. (1999) concluded that utilization of spun urine (centrifuged deposits) in conjunction with vaginal fluid examination improves the detection rate of Trichomonads. This parasite is also a cardinal parasite of male urogenital tract and is an important sexually transmitted infection. This flagellate can be seen in urinary sediments from males as well (Gardner et al. 1986; Kaydos-Daniels et al. 2004). The flagellate can be picked up with the help of its pear shaped morphology, characteristic motility and flagella. This flagellate may increase the risk of HIV transmission (Sorvillo and Kerndt 1998; Kaydos-Daniels et al. 2004), also it has been found to be associated with carcinoma prostate (Sutcliffe et al. 2012). Thus presence of this flagellate parasite essentially calls for treatment of both the partners. Examination of urine within 1 h of collection ensures highest possibility of detecting these parasites.

Urinary *balantidiasis* has been reported rarely and mostly in immunocompromised patients like diabetics and renal transplant recipients (Karuna and Khadanga 2014; Bandyopadhyay et al. 2013). In this study there were two cases of ciliate infections, one was a psychotic young male patient with *B. coli* infection and the other one was a geriatric female with probable infection with *Chilodonella* spp. Urine routine examination and cytology helped us in differentiating the latter from *Balantidium* spp., *Buxtonella* spp., *Colpoda* spp. The trophozoites of this ciliate were much smaller, had an asymmetrical oval shape with a pointed beak, the ciliary tuft was seen at one end, and had an oval macronucleus. The body of the ciliate had right arched ciliary kinetics and left straighter ciliary kinetics forming the characteristic shape of *Chilodonella* spp. (Mitra and Haldar 2004). These ciliates have a slightly convex dorsal surface which does not have cilia and a flat ventral surface which has cilia and hence the movement is along one surface unlike the rotatory motion of *Balantidium* spp. Researchers suggest that Chilodonellids are capable of forming cysts. A cyst like structure was seen in this case. *Balantidium* spp. in contrast are much bigger in size, oval shape, have short and fine cilia covering the entire body, and sausage shaped macronucleus. The comparative

morphology and motility of these ciliates have been depicted in tabular form (Table 1). Earlier it was thought that only *B. coli* is pathogenic to humans but with the increasing case reports on extraintestinal balantidiasis (Karuna and Khadanga 2014; Bandyopadhyay et al. 2013, Majumdar et al. 2014) and infection by some ciliates morphologically different from *Balantidium* (Costache et al. 2011), the diagnostic species confirmation becomes essential with molecular methods (Pomajbikova et al. 2013; Schuster and Ramirez-Avila 2008). However it was not possible in this study due to the lack of this facility presently at our institute.

Majority of the patients in this study had urinary complaints like increased frequency and dysuria (27/33). Pain

abdomen (2), psychosis (2), fever (2) and joint pains (1) were some of the additional complaints. Two patients had come for a routine checkup and *T. vaginalis* was detected incidentally (2/33). One each had presented with eczema (1/33), back pain (1/33), pruritus and dyspareunia (1/33).

Out of the three cases displaying characteristic planoconvex eggs of *E. vermicularis*, one was a 6 year girl and the other two were 7 year old females presenting with urinary complaints. Urinary tract infection, pelvic inflammatory disease of female genital tract (Avram et al. 1984; Ok et al. 1999; Patel et al. 2015) and rarely of male genital tract (Zahariou et al. 2007) has also been reported earlier with Enterobiasis. Also enterobiasis has been rarely

Table 1 Morphological comparison of *Balantidium* like ciliates with the cases in this study

Species	<i>Balantidium</i> (Bandyopadhyay et al. 2013)	<i>Buxtonella</i> (Pomajbikova et al. 2013)	Colpoda (Costache et al. 2011)	<i>Chilodonella</i> (Mitra and Halder 2004)	Indexed case 1 (young male) (<i>B. coli</i>)	Indexed case 2 (geriatric female) (<i>Chilodonella</i>)
Shape	Oval, Broad posterior end and sharp anterior end	Somewhat round shaped	Kidney shaped	Asymmetrically oval forming a beak at left end	Oval, broad posterior end and sharp anterior end	Asymmetrically oval forming a beak (vase like)
Size	30–300 μ	60–140 μ	40–45 μ	28–70 μ	60 μ	30–40 μ
Cilia	Short and fine somatic cilia covering the entire body, cilia around the vestibule appear longer	Short fine cilia throughout and along the sulcus	Short cilia present throughout the entire body and along the cytostome	Three row of cilia at one end: peroral, post oral and circumoral. Ventral surface has cilia	Short and fine covering the entire body, cilia around the vestibule appear longer	Cilia present at one end and along one surface
Ciliary bands	Not prominent	Not prominent	Not prominent	Prominent, Left straight ciliary bands and right arched ciliary bands. Ventral surface is flat and has row of cilia	Not prominent	Left straight ciliary bands and right arched ciliary bands
Location of cytostome; cytopharyngeal groove	At tapering anterior end;	At one end, very near to the cytophyge. Very long vestibular groove running from one end to another forming a sulcus	A vestibulum is located at 1/3rd to half the distance along the inner curvature which leads to cytostome	At the top a vestibulum is present which leads to cytostome; present (with rod like apparatus in some species)	At tapering anterior end	Present near anterior end
Macronucleus	Sausage shaped	Oval shaped	Spherical	Round	Stained deposits not available	Round
Common hosts	Humans/pigs	Cattle	Invertebrates like mollusks; rare cases in humans	Fish	Human in this case	Human in this case
Motility	Swimming, rotatory, spiraling	Rotatory	360 degree motility	Moves along the ventral aspect which has cilia	Swimming, rotatory, spiraling	Movement was along one aspect

reported from eyes (Babady et al. 2011), nose, salivary gland and lungs.

Strongyloides stercoralis in urine has been reported in an immunocompromised patient (Pasqualotto et al. 2009). It has also been reported in cervical smear (Avram et al. 1984, Murthy et al. 1994). The absence of lateral alae, muscular esophagus and characteristic eggs ruled out the possibility of *E. vermicularis*.

Spermatozoa attached to a pus cell or epithelial cell can resemble a flagellate like *Trichomonas*. Numerous bacilli attached to epithelial cell or pus cell can resemble a ciliate parasite like *B. coli*. Cotton or gauze twigs used to clean slides can resemble nematodes like *Strongyloides*. Starch granules in urine can resemble eggs of *H. nana*. *H. nana* is an intestinal cestode causing tapeworm infections in humans with worldwide distribution. No vector is involved and infection is by feco-oral transmission (Cheesebrough 2009). In this series, urine examination of a 3 year old girl presenting with UTI revealed starch granules due to fecal contamination, which resembled the eggs of *H. nana*. A repeat stool and urine examination confirmed the presence of starch granules rather than parasite ova. The urine culture revealed *E. coli* infection.

The urinary parasite *S. hematobium* is an infection in humans by parasitic blood worm, and fresh water gastropod snails are the intermediate host vectors (Morenikeji et al. 2009). The prevalence of this parasite has been reported as high as 23.2% in a study from south-west Nigeria; however it is rarely found in Indian set up (Kali 2015). Sometimes microfilaria can be detected in the urine (Ahuja et al. 2012; Vankalakunti et al. 2008). The filarial infections are more common in coastal regions of India and are not very common in central India.

To conclude, increased frequency, dysuria, pyuria and hematuria as evident in this study, indicates a high possibility of finding parasites on urine evaluation in such patients. A high index of suspicion and a meticulous search for the parasite in centrifuged urine sediments is thus indicated. In addition, identification of morphology with associated motility in vital preparation is an economical and convenient tool in clinical pathology practice.

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Collection of patient details, Digital Photography. DB: Expert opinion and critical review of the manuscript.

Compliance with ethical standards

Ethical approval Yes; all investigations were done as part of the patients' management protocol, and no patient was subjected to any procedures or investigations for the sake of this study. Approval from the competent authority of the institute has been taken.

Conflict of interest None.

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