Review Article

Parasites observed in urine sediments: A Rare but Convincing Truth

ABSTRACT

Aim: To reveal the urgency to understand and augment the recognizance of the possibility existence of parasitic materials on cytological evaluation of urine.

Discussion: Urine is always contemplated as an ideal diagnostic specimen due to its direct and easiness to collect that surely non-invasive. Three common parasites that can be found in urine are *Trichomonas vaginalis, Schistosoma hematobium* and microfilaria of *Wuchereria bancrofti*. Other parasites and parasitic ova may also be seen in urinary sediments as a result of fecal or vaginal contamination. Urine Parasitological analysis embraces a wide expansive areas of tests, which comprise an assortment of physico-macroscopic analysis, microscopic analysis on cells and Parasite-bacterial appearance, parasite and microbe cultures, chemical tests, and even can be extended up to molecular identification. Importantly, from urine samples, a confirmed Parasitological diagnosis (and also Microbiology and other kind of disease) can be made through definite tests.

Conclusion: In the context of allegation of parasite as an etiological agent, thorough morphological detection via careful sediment microscopic analysis helps in making early and correct diagnosis in most cases.

Keywords: noninvasive, macroscopic, microscopic, cells, Parasite, bacteria, cultures

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1. INTRODUCTION

Urine can be considered as a window to the body's inner workings and might reflect~~ed~~ certain different conditions, even diseases [1]. Urine is always contemplated as an ideal diagnostic specimen due to its direct and easiness to collect that surely non-invasive [2,3]. Urine as an entity is an unstable liquid; it underwent adjustment in quality contents, e.g., metabolites, that immediately started after it is urinated through the process of micturition [4]. Unlike blood whose quality/composition and quantity/volume are rigidly maintained, urine is not subjected to homeostatic mechanisms [5]. Precise and accurate collection, storage, and handling are crucial in ~~to~~ maintaining the samples ~~sample's~~ [1-5]. In the context of “incidental” means that it is liable to happen as a consequence of an infection that occur inside or outside the genitourinary tract.

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in the context of urinalysis, if there is a suspicion that the cause is an infectious agent, then there are two possibilities: (1) the infection indeed occurring in the urinary tract and the organism can be seen on direct examination or (2) the infection occurs elsewhere but the metabolites can be found in the urine because of: (2.1) accidental contamination, e.g., the ova of *Enterobius vermicularis*, and (2.2) the possibility that these metabolites or other

parasite product/consequences are dissolved in water and not in lipid, so excretion take place via the kidneys, or in other words that this type of infection indirectly can be found in the urine, e.g. black water fever.

The aim of this review is to reveal the urgency to understand and augment the recognizance of the possibility existence of parasitic materials on cytological evaluation of urine.

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2. PREPARING THE URINE SEDIMENT

For decades, the manual of urine sediment analysis was a gold standard in laboratory work regarding urine based clinical sample. In most laboratories, a regular light microscopy of pristine, centrifuged native urine is even now an integral part of ordinary laboratory work. However, regarding all-inclusive protocols, primarily in the preanalytical stage, scarcely differ between laboratories. Actually, there is no reference method for urine sediment microscopy [6].

The preanalytical stage is the most vulnerable part of laboratory process accounting for up to 75% of all laboratory errors [6,7]. The preanalytical phase is comprised of several sub-phases: instruction for conducting the test, patient preparation, sample collection, sample preservation including its transport and preparation of sample for examination under the microscope. Even though the laboratory is not directly involved in all those steps, laboratory staff is responsible for their correct execution [8].

Patient Preparation. In the very ideal condition, patient should receive instructions on the following: (1) must refrain from strenuous physical exercise approximately 48 hours prior to sample collection, (2) must refrain from sexual intercourse approximately 24 hours prior to sample collection, (3) Not to give urine samples during their menstrural period (for women), (4) must refrain from drinking too much on the morning of the collection and the night before (approximately 200 ml of fluid after 22.00 and no more until sample collection with assumption the sample will be taken at 8 am; drinking excessive amounts of water may cause inaccurate results. One or two extra glasses of fluid, which can include juice or milk if ~~your~~ their diet allows, is all that is needed at the day of the test. But patient must also be informed that prolongation of time to conduct the sample collection while they already in restricted drinking might also interfere the result of the test).

For Parasitology examination, timing of the sample collection usually random specimen, means that whenever the patient comes to the laboratory, the sample can be immediately taken and collected. Manner while the sample collected is should be as follows: (1) carefully washing the hands, (2) for female patient, spreading the labia or for male patient withdrawing the glans of the penis, (3) carefully cleaning the urethra orifice with clean disposable wet towelette and then dry-wipe with clean paper towel, (4) urinating in this condition: let the first portion pass into the toilet and then carefully without breaking the stream collect and catching the mid-stream urine in the correct container (at least 50-100 ml, opening of at least 5 cm diameter) and avoid touching the inside part of the container and immediately closed the container lid as soon as the desired amount of urine is collected. If the sample comes from a catheter of an individual with a serious illness, it needs more careful handling, e.g., single catheterizations are discouraged, ~~if~~ when taking samples from indwelling catheters never take the sample from the collecting bag and it is preferable to block the passage of urine for approximately 1 hour and then aspirate the sample from catheter. Suprapubic aspiration is encouraged if all the supporting device and resources are available.

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Sample Preservation and Transport. Ideally, the sample should be examined as soon as possible, or at least within one hour after voiding [1,9]. If a delay is expected, the sample should be refrigerated [1,8-10]. The addition of fixatives is not regularly conducted [7].

Sample preparation. Urine sediment preparation is undertaken to identify cells, casts, crystals, and/or microorganisms [4,6-10]. Sample preparation are as follows: (1) mix the urine sample by gently inverting the container; (2) using a disposable plastic pipette, transfer 5 ml of urine to a conical tip centrifuge tube. In practice, there may be 1ml or 5ml conical tubes to use. Processing is ~~was~~ done by centrifuging at 2500 rpm for 10 min or 400 g for 5 min, depending on tool's availability. Whatever type and size of centrifuge, always remember to balance it correctly and secure the lid, (3) Once the centrifuge has stopped, remove the tube, and remove the supernatant (free fluid at the top of the sample) with a plastic pipette or by decanting; (3) Keep approximately 0.5 ml of supernatant in the tube. Take care not to disrupt the sediment "pellet" whilst removing the supernatant and then wet unstained sediments obtained. (4) In our laboratory, we usually add one drop of the physiology saline to the tube, using a pipette then followed by re-suspending the sediment in the supernatant by gently swirling or "finger-flicking" the tube; (5) use a pipette to transfer one drop of the reconstituted sediment to a microscope slide and then slowly cover it with a coverslip over the sample and the sample ready to be examined; (6) Place the covered slide on the stage of the microscope and perform examination using the low power (x10) first and then the high power (x40) lens.

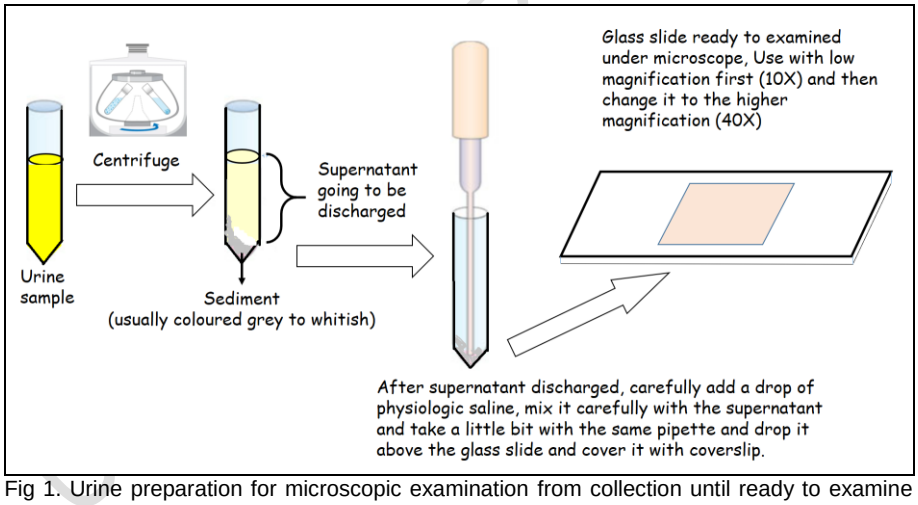

Fig 1. Urine preparation for microscopic examination from collection until ready to examine glass slide. Careful microscopic inspection of urine sediment can reveal the presence of cells, casts, crystals, and/or organisms.

3. PARASITIC AGENT OF THE URINARY TRACT, DIRECTLY OR INDIRECTLY

Parasitism is a complex condition of relationship that can affect biodiversity, including each acquaintance alliance in which a species hang on upon other species for its well-being and actuality [18]. This unique relationship may occur in rhythms as a brilliant strategy for intra-host survival and also inter-host transmission [19]; and provide the parasite a certain milieu

that can only exists for only certain period (temporary) or on contrary lasts as long as the organism is still alive during its attack on its vulnerable host (permanent). The harboring organism, known as the host, unfortunately may exhibit no harmful effects in mild subclinical infection, but can also be indicate countless functional and organ-based dysfunction [20].

The endemicity of a parasite in a certain geographic area depends upon the presence and daily activities/habits of its appropriate and ready to serve host(s) [21] and also upon tranquil get away from their intermediate, paratenic or even definite host [22]. Parasites with simple life cycles are most likely to have a more cosmopolitan distribution than those with complicated life cycles, e.g., parasitic Crustacean [23]. Climate [24], e.g., the rises in temperature might disturb the life cycles of parasites, which may straightly shift the abundance or prevalence of the organism is within certain area, considering most parasites need a temperature-dependent developmental baseline, either when they are inside their host or living freely in the environment, as well as socioeconomic [25] conditions of the host and the community in general affect the distribution of human parasites.

Poor practice of personal hygiene [26] and communal sanitation [27] that combined with truncated standards of living and incomprehension or ignorance facilitate the spread of parasitic diseases [28]. The life cycle of a parasite and its adaptation to external condition may involve survival and development outside their host [29], e.g., live freely in the environment, and inside a single or even more hosts; there is rhythms in strategies for within-host survival and between-host transmission [30]. Parasite's life cycle may be relatively simple or it may be complicated, with numerous morphological forms and developmental stages and this is related to its evolutionary ecology [31].

With the world nowadays supporting massively type of transnational and transcontinental migration through every mass transportation, these human movement bring along some diseases (including parasites) which are normally endemic in their home countries of origin, to the new countries [32]. Similarly, environmental changes that result from development of water resources, global warming, growth and migration of population can facilitate the spread of parasites [24,29,30].

the parasite may be in an inactive state while they are in the stage of resistant eggs [33] or cysts [34]. When taken up by an appropriate host it may undergo adaptive and active growth with even establish metamorphosis [35]. As the life cycle of certain organism with parasitic behavior becomes more complex, the chances of their survival clearly decrease [31], but highly developed reproductive organs and multiplication at some stage of the parasite's life cycle offset the hazards of a complex life history [36]. Host–parasite interactions often affect the population dynamics of the two antagonists.

In the external environment or outside host's body, the effort to conduct analysis on urine sample holds a wide expansive areas of tests, which comprise an assortment of physico-macroscopic analysis, microscopic examination on cells found in urine and also the possibilities parasite-bacterial spotted, and can be further processed to be cultured for parasite and microbe, certain chemical tests, and even can be extended up to molecular identification [1,37]. Primarily, from urine samples, a firm and confirmed Parasitological diagnosis (and also for Microbiology and other type of ailment) can be justified by using certain tests or analysis [38].

The most important parasitic diseases involving the genitourinary system in humans are helminth based, named *Schistosoma haematobium* [11]*, Echinococcus granulosus* [12] and *Wuchereria bancrofti* [13]. Non-helminthic parasites that can infect the genitourinary tract including protozoans like *Trichomonas vaginalis* [14] and *Entamoeba histolytica* [15].

Another aspect of this is the infection occurs elsewhere but its metabolites can be found in the urine, e.g. the blood protozoan *Plasmodium falciparium* [16]. Last but not least, another issue with urine as a sample source is the risk of contamination during collection or handling; and that source can be fecal or vaginal contamination [17].

3.1 *Schistosoma haematobium* and Schistosomiasis

Schistosoma hematobium together with *S. mansoni* are the primary schistosomal organisms that affect the kidney. This urinary tract helminth causes prominent glomerulonephritis, leading to nephrotic syndrome [40]. *S. haematobium* are present in the venus plexus of the bladder, expelling eggs in the urine. *Schistosoma hematobium* responsible for producing obstructive uropathy, leading to prominent interstitial inflammation with mononuclear cells and fibrosis. Schistosomal eggs (notably from *S. mansoni, S. hematobium,* and *S. japonicum*) may be localized to the kidney and may incite a granulomatous inflammatory reaction. The eggs of *S. hematobium* (with characteristic terminal spines) are not acid fast [11,39].

Definitive diagnosis of *S, haematobium* infection in adult patients is a truly important challenge from clinical perspective. Chronically affected adults could pass certain number of eggs in the urine, which are often missed due the characterization of momentarily passage of the egg [41].

The characteristic clinical presentation is terminal hematuria, usually associated with increased frequency of micturition and dysuria. Diagnosis is made by finding the characteristic ova in the urine [39-42].

3.2 *Echinococcus granulosus* and Hydatid Cysts

Human cystic echinococcosis (CE) is a chronic illness, which was actually zoonosis, formed by the flatworm larvae of the helminth *Echinococcus granulosus* [43,44]. Epidemiologically, its endemicity can be found in many regions of the world and the pattern of its distribution has not changed over the last twenty years [43].

In humans, CE cysts primarily affect the liver [45] and pulmonary [46] system, but can also affect the kidney [47]. However, the clinical manifestations of renal CE can be exquisite, sometime only mild hematuria in appearance, and for that reason even healthcare professionals sometime dealt with uncertainty and frequently overlook renal CE in differential diagnosis [48].

Genitourinary echinococcosis is actually an uncommon cyclo-zoonotic ailment [49]. The solely trademark sign of genitourinary echinococcosis is the presence of hydaturia (presence of daughter vesicles in urine) [44], but this only found in 10%–20% of the cases. Reports suggest that left renal involvement is more common than right, and from the perspective of Anatomy, that happened probably due to the smaller size of renal artery [44,47]. Renal lesions are usually found in the cortex and can be uni- or multilocular [50].

3.3 *Wuchereria bancrofti* and other filarial nematodes

In 1866, Wucherer discovered microfilariae, by the identification of threadlike worms, in clinical specimen urine [51]. Chyluria is an uncommon medical condition resulting from an abnormal communication between the abdominal lymphatic system and the urinary tract, perhaps due to rupture of dilated abdominal lymphatics into the urinary excretory system, which then results in

the presence of chyle in the urine, making it appear milky white [52]. Chyluria is caused by discharge of intestinal lymph (chyle) into the renal pelvis and subsequently into urine. Chyluria designates the existence of an uncommon communication between the intestinal lymphatics and urinary tract; and this will result in the origination of lymphatico-urinary fistulae [53]. Once such a fistula is formed, milky white urine is passed [52,53], which may be discharged intermittently or continuously [54]. In chyluric patients, accompanying hematuria is caused by the rupture of minute blood vessels into the urinary tract during the formation of lymphaticourinary fistulae [55]. Patient presented with chyluria can also be found with intermittent hematuria [54,55].

The adult filarial worm causes lymphangitis, lymphatic hypertension and valvular incompetence. Thus, shedding of microfilariae in urine is probably determined by local factors like lymphatic blockages caused by scars or tumors and damage to vessel wall, caused by inflammation, trauma or stasis [52-55].
Considering that this disease is endemic, if a doctor treating a patient with a clinical condition of a combination of chyluria and hematuria and who comes from an endemic area, then filariasis must always be taken into account first.

3.4 *Trichomonas vaginalis* and Trichomoniasis

Vaginal trichomoniasis is an exceptionally prevalent infection which has been related with acquired human immunodeficiency virus accretion (where it augments the likelihood of HIV acquisition by 50%) [56], other type of sexually transmitted disease [57] and adverse birth outcome, e.g., preterm birth [58].
Women with complaint suspected for *T. vaginalis* infection can be ~~were~~ screened by wet-preparation (wet-prep) microscopy and culture and for the presence of *T. vaginalis* DNA by specific PCR of vaginal and urine specimens [60].
The positivity of trichomonosis can be ~~was~~ defined as the detection of *T. vaginalis* by direct microscopy and/or culture from either vaginal samples or urine.

3.5 *Plasmodium falciparum* and Black Water Fever

P. falciparum infection is detectable by PCR method by using human urine and saliva samples [61]. Subject to further refinement of extraction technique and amplicon yields, large-scale malaria parasite screening and epidemiological surveys could be possible without the need to collect blood and use of needles or sharps.
Blackwater fever (BWF) is a collection of clinical syndrome occurring as a complication of severe malarial infection. This BWF portrayed by intravascular hemolysis, hemoglobinuria, and acute renal failure in patient exposed to the hemoflagellata *P. falciparum* [62] and, to some extent, in malaria (+) patient who were exposed to medications like quinine [64] or mefloquine [62].
The exact pathogenesis of quintessential BWF remains fuzzy. The mechanism leading to demolition of infected erythrocyte actually can be immunologic and non-immunologic, leading to massive intravascular hemolysis [62,63]. Malaria is also endemic to some region of the world, so adequate history taking of travelling or in refugee settings, coastal areas,

urban areas and river basins, all of them fortify the essence of investigations into how human migration across the globe may be interfering with malaria infection incidence [65].

4. CONCLUSION

With increased concern regarding the frequency of clinical condition of dysuria, pyuria and hematuria as evident in this review, designates an elevated possibility of revealing 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.

CONSENT

Not needed

ETHICAL APPROVAL

Not needed

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