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SCREENING OF URINE CYTOLOGY FOR CHANGES DUE TO LONG TERM EFFECTS OF URINARY SCHISTOSOMIASIS IN MANAGIL CITY, GEZIRA STATE, SUDAN

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ABSTRACT: *Schistosomiasis or bilharziasis is water-borne flatworms' affects approximately 200 million people worldwide. This cross sectional laboratory based study to screen urine for cytological changes in urinary Schistosomiasis patients, in individual previously diagnose. From 89 patients (88 male and 1 female) represented the study group voided urine samples collected in sterile container, centrifuged and prepare as monolayer smear, fixed before air dying in 95% ethanol and stain by pap stain. The results was*: *The majority of study group 36/89 (40%) were workers and 53/89 (60%) students. Most affected group age range (11-20) years. Microscopic examination demonstrate urine crystals 24/89 (27%) samples, while 65/89 (73%) of samples were negative.11/89 (12%) contains yeast cells and 78/89 (88%) were negative. 5/89 (6%) contains urine cast and 84/89 (94%) were negative. Positive cases as follow: 3/5 hyaline cast and 2/5 cellular cast. Most of the smears were hypocellular (3-5 cells/field). Cellularity according to the type of cells: normal urothelial cell present in (9) samples (10%) intermediated epithelial cells present in (8) samples (9%). Corpora amylacea present in (5) samples (6%). Condyloma acuinatum present in (1) sample (1%). Cells show Koliocytosis similar to that of cervical infection. One patient has hematouria (1%) with elements indicate active cystitis. In addition to this sample another (16) samples to give total 17/89 that account (19%).Follow up is necessary to avoid untreated complications that may develop later on.*

KEYWORDS: Urinary Schistosomiasis, Schistosoma hematobium, Urothelial cells, Urinary cast, Cystitis.

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INTRODUCTION

Schistosomiasis, also known as, bilharzia, bilharziasis, or snail fever, affects approximately 200 million people worldwide (WHO. 2010). WHO has placed schistosomiasis as the third most devastating tropical disease, following malaria and intestinal helminthiasis (WHO-TDR. 2010)

In Sudan, since the year 1919, the disease been discovered in the northern part of the country and later it reported from different parts including Eastern and Western Sudan, Lake Nasser area, and many agricultural schemes such as Gezira Scheme, Rahad Scheme and Gunaid Sugar Cane Scheme. (Suleiman Y, et al. 2017)

There are five species of Schistosomiasis. These water-borne flatworms or blood flukes are schistosomes. The most common are Schistosoma mansoni, S japonicum, and S haematobium. The rarer forms are S intercalatum and S mekongi. S mansoni occurs in Africa, the Caribbean, South America, and the Eastern Mediterranean. S japonicum and S mekongi found in Southeast Asia and the Western Pacific. S intercalatum is endemic in central Africa and S haematobium occurs throughout Africa and the Eastern Mediterranean. S haematobium affects both the urinary and reproductive tract systems, whereas the four other species affect the hepatic and gastrointestinal systems (Schmitt I. 2006)

The infection of S. haematobium still leads to some form of abnormalities, Mortality may occur from granulomatous inflammation complications (Hams E, et al. 2013). Renal insufficiency (Da Silva G B, et al. 2013), possible bladder cancers (Botelho M, et al.2017), and ulceration or depletion of the vesicle, as well as the ureteral wall (Hams E, et al. 2013)

Praziquantel is the recommended treatment against all forms of schistosomiasis. It is effective, safe, and low-cost. (WHO.2011)

Rationale:

Schistosomiasis is most common disease next, the malaria, worldwide there is high morbidity as consequence of Schistosomiasis. This study conducted in Al Managil city which known as suspected area for high disease incidence, because it's location in Gezira agriculture project, one of endemic Schistosomiasis areas in Sudan, so this study conducted to fill the gap of information and to provide prospective about the Cytological changes in urinary Schistosomiasis in long term infection patients whose already diagnosed and obtain the course of treatment and don't attain for regular follow up.

RESEARCH OBJECTIVES

General Objective:

To screen urine cytology for changes due to long term effects of urinary Schistosomiasis patients in Managil city. Gezira state. Sudan.

Specific objectives:

- To detect cellular changes that would be present in chronic urinary Schistosomiasis patients.
- To detect an abnormal findings other than cytological changes.

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- To identify the technical ability of conventional method of urine cytology in demonstration of changes associated with urinary Schistosomiasis.

Scientific background:

The cytological examination of a urine specimen is a simple and inexpensive method of assessing patients who present with haematuria (blood in urine). However, haematuria is a common presentation for a variety of non-malignant conditions (Behdad S. 2011)

Specimen type:

Voided urine:

The collection method of choice for screening for urological disease ،early morning urine specimens provide poor samples for cytologic examination. Rapid transport to the laboratory recommended. If a short delay is inevitable, the container may place in a refrigerator. For longer delays, prompt fixation can achieved by collection of 50-100 mL of urine into an equal amount of 50% alcohol. Sensitivity of urine cytology increases with the number of specimens examined (Gray W and Kocjan G. 2010)

Catheterised specimens:

May submitted if clinically indicated. Because of cellular changes present in catheterised specimens, it is essential that the clinician indicate the nature of the sample (Gray W and Kocjan G. 2010)

Bladder washings:

If clinically indicated this method of collection may be superior to voided urine. Disadvantages are the same as for catheterised specimens. Again, the laboratory must informed of the method of specimen collection for accurate interpretation (Gray W and Kocjan G. 2010)

Brushings:

Adisposable or non-disposable brush may introduced through a cystoscope. The sample is brushed directly onto slides which can be alcohol fixed or air-dried depending on the laboratory protocol (Gray W and Kocjan G. 2010).

Benign findings:

Normal urothelial cells have bland nuclear chromatin, uniformly round nuclei, inconspicuous nucleoli, and frothy cytoplasm (Dorothy L, Rosenthal. 2005). Reactive changes may seen in a variety of conditions in addition to lithiasis, radiation therapy, chemotherapy and viral effect. The Atypia in reactive conditions is commonly secondary to inflammation and/or cellular degeneration (Gray W and Kocjan G. 2010). Reactive/inflammatory changes in urothelial cells are similar to those of all epithelial cells, i.e., accentuated nucleoli, slightly coarsened chromatin, round nuclei and a variably increased nuclear cytoplasmic (NC) ratio. In contrast, cells from low-grade urothelial carcinoma have oval nuclei, indiscernible nucleoli, and high NC ratios (Dorothy L, Rosenthal. 2005).

Most infectious agents are not obvious in voided urine or washings, but occasionally trichomonads, evidence of polyomavirus (decoy cells), Herpes simplex virus, cytomegalovirus

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(CMV) or human papillomavirus (koilocytes). Other viruses that have been identify in urinary tract specimens include measles virus and adenovirus (Gray W and Kocjan G. 2010).

Schistoma ova found rarely in our practice, but should sought when extensive squamous metaplasia seen. Renal tubular epithelial cells are usually so degenerated by the time they reach the bladder that they resemble histiocytes. They are usually few in number, unless there is intrinsic renal disease affecting the tubules. Cellular casts preserve the cytomorphology these cells, and are important to report (Dorothy L, Rosenthal. 2005).

Benign Non-epithelial Elements

Cytology reports should include not only cellular elements, but also other features that have clinical significance. These include casts, crystals, inclusions, and ejaculate which may include seminal vesicle cells, not to be mistaken for neoplastic cells (Dorothy L, Rosenthal. 2005).

Polymorphonuclear leukocytes, necrotic debris, histiocytes, degenerative changes in urothelial cells, urothelial hyperplasia, and often-reactive urothelial Atypia (Bibbo M and Wilbur D.2015)

Bacterial cystitis is usually caused by faecal flora, Gram-negative organisms such as Escherichia coli (80% of infections), Proteus, Klebsiella and Pseudomonas aeruginosa. Bacterial infections occur mostly in adult females but also may occur in patients with urinary tract obstruction, such as stones, or men with prostatic hypertrophy, and patients with nerve damage. In most cases, voided urine specimens show abundant bacteria and acute inflammation. Urothelial cells typically are few and are poorly preserved, showing a hyperchromatic, small nucleus and frayed cytoplasm. The absence of nuclear detail is the key to not making a diagnosis of high-grade urothelial carcinoma (Gray W and Kocjan G. 2010)

The most common fungal form seen in a voided urine specimen is Candida, which in women is most often secondary to vaginal contamination; these preparations show squamous cells and bacteria, in addition to Candida, and the urothelial cells are greater in number and non-reactive in appearance. Patients may present with Candida cystitis, although other fungi, including Histoplasma , Aspergillus , Cryptococcus and Blastomyces have been reported (Gray W and Kocjan G. 2010)

Parasitic infections of the urinary tract include Trichomonas and Schistosoma. Trichomonas vaginalis usually is a contaminant in women and the organisms seen in association with intermediate squamous cells and acute inflammation. Schistosomiasis of the bladder is most common in the Middle East and mainly caused by Schistosoma haematobium. The eggs of Schistosoma may observed in urinary tract specimens Schistosomal infections are characterised by exuberant acute inflammation, blood, squamous metaplasia, and marked reactive urothelial and squamous cell changes. Schistosomal infections are a risk factor for squamous cell carcinoma of the bladder. Other parasites infecting the urinary tract also accompanied by a marked inflammatory response (Gray W and Kocjan G. 2010)

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PREVIOUS STUDY

The study of Cecilia Smith *et al* in 2021 in which urine samples (160) were screened for the presence of S. haematobium ova. Smears stained with the Papanicolaou method were evaluated using light microscopy to determine the cell populations. A high prevalence (39.9%) of urinary schistosomiasis and haematuria (46.9%) found among the participants. Polymorphonuclear cells, normal andreactive urothelial cells and lymphocytes were characteristic of S. haematobium infection. Squamous metaplastic cells (SMCs) were found in 48% and 47.1% of participants who have had past or current S. haematobium infection respectively, but were not found in participants who had no exposure to S.haematobium (Smith Togobe et al. 2023).

Another study made by Patience B and his group 2019 in (Accra) Ghana, a study was conducted under the title Cytological and Wet Mount Microscopic Observations Made in Urine of Schistosoma haematobium-Infected Children: the study was carrid out to identify microscopic and cytological abnormalities in the urine deposits of S. haematobium-infected children, the study was achieved in 367 urine samples were collected from school children in Zenu and Weija communities, All the samples were examined microscopically for the presence of S. haematobium eggs, after which the infected samples and controls were processed for cytological investigation. They found that S. haematobium ova were present in 66 (18.0%) out of the 367 urine samples. Inflammatory cells (82%, 54/66), hyperkeratosis (47%, 31/66), and squamous cell metaplasia (24%, 16/66) were the main observations made during the cytological examination of the S. haematobium-infected urine samples (Patience B, Tetteh-Quarcoo et al. 2019)

METHODOLOGY

Study Design:

This study was Cross-sectional laboratory based study

Study Area:

Managil City in Gazira State. Sudan.

Study duration:

From January2023 to September 2023.

Study population:

Individuals who were previously diagnose with urinary Schistosomiasis irrespective of the age and gender.

Inclusion criteria:

Individuals with urinary Schistosomiasis.

Exclusion criteria:

Individuals with urinary disease other than Schistosomiasis.

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Sampling and sample size:

Random urine from 89 patients represent the study group.

Methods of Data Collection:

The data collected from questionnaire.

Ethical considerations:

Ethical approval:

The approval obtained from Faculty of Medical Laboratory Sciences university of Managil, Department of Histopathology and. Cytopathology.

Ethical permission:

The permission obtained from the Faculty of Medical Laboratory Sciences, Managil University, according to the restrictions of the Ministry of Health.

Ethical consent:

After verbal approval for the patient either to accept or refuse to share in the study, the consent that collected from the individuals or master under privacy and confidentiality and will not use for any purposes rather than this study.

Laboratory work

The sample was collected in sterile urine container, 5 ml of the placed in centrifuge tube, centrifuged at 1500 rmp for 10 minutes after which the supernatant was decayed leaving about 1.5 ml. The volume was resuspended and cytocentrifuged 3-5 drops per slide in order to prepare monolayer smear , The smear slides were wet fixed with 95% ethanol for 15 minutes before air drying then stain with pap stain (J Y Kim H J Kim. 2014)

Staining procedure:

- Take the smear to water (hydration), dip for 2 minutes in the following solution of ethanol 80% (v/v), 70 %(v/v) and 50 %(v/v) respectively.
- Rinse in water for 1 minute.
- Stain in Harris's hematoxylin for 5 minutes.
- Rinse in water for 2 minutes,
- Differentiate in 0.5% aqueous hydrochloric acid for 10 seconds approximately.
- Rinse in water for 2 minutes
- Place under running tap water for 2 minutes, rinse in water for 2 minutes,
- Dehydrate through alcohol solution 50%(v/v), 70% (v/v), 80%(v/v) and 95% (v/v) by dipping in 2 minutes for each solution,
- Stain in OG 6 for 2 minutes, rinse in 95% alcohol for 2 minutes
- Stain in EA 50 for 3 minutes, rinse in 95% alcohol for 1 minute.
- Dehydrate in absolute alcohol
- Clear alcohol using xylene for 2 minutes, and mounting medium (Suvaran S K, et al.2019)

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Statistical analysis:

The data analyzed by using Statistical Package for Social Sciences (SPSS) Computer Software and Microsoft excel.

RESULTS

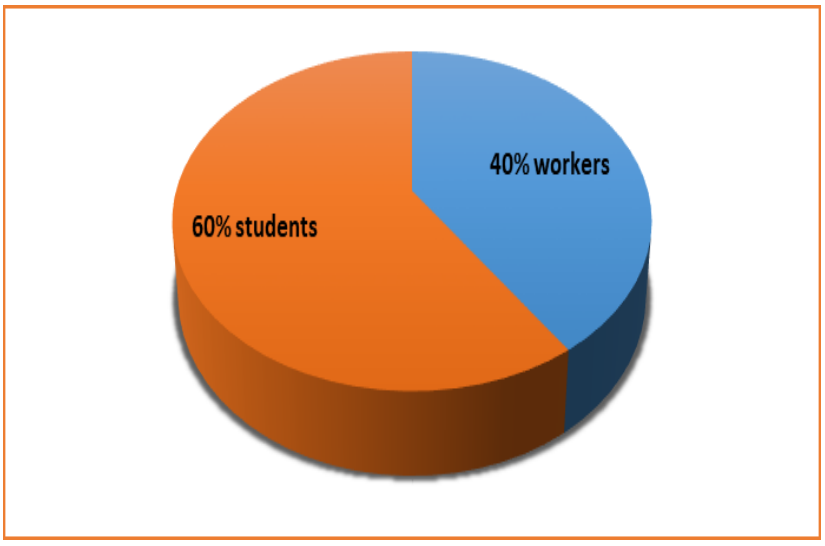

Figure (1) shows the distribution of study population according to occupation. 60% patient were students and 40% were workers.

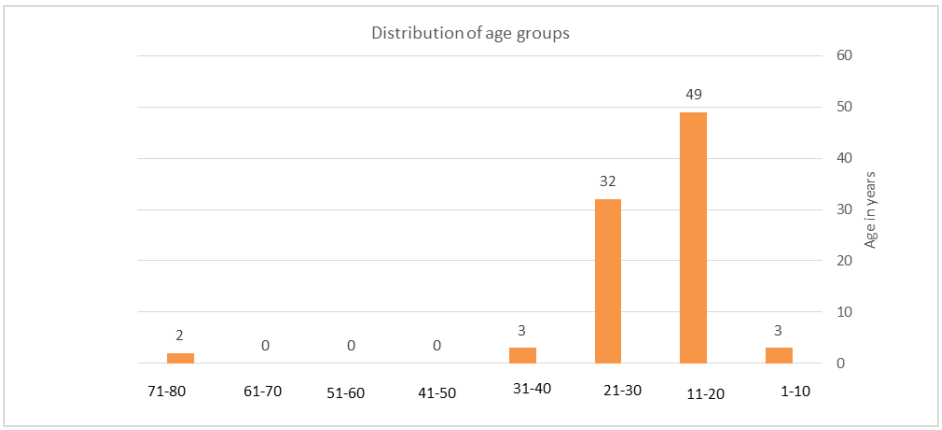

Figure: (2) Distribution of age groups.

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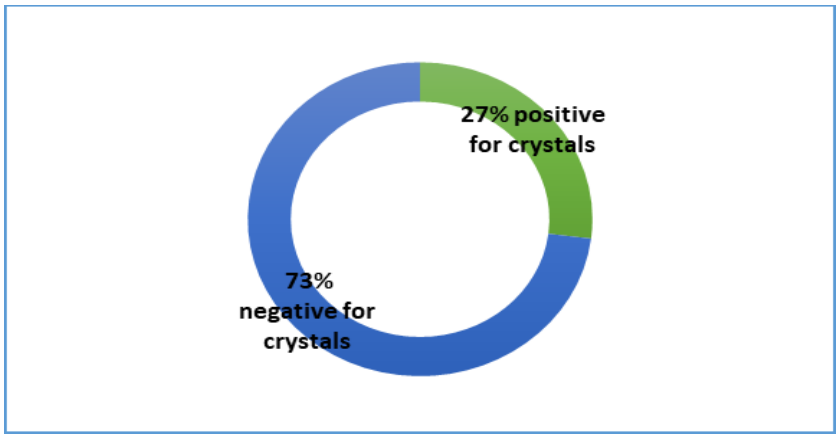

Figure: (3) Presence of urine crystals: 27% positive of crystals and 73% negative of crystals.

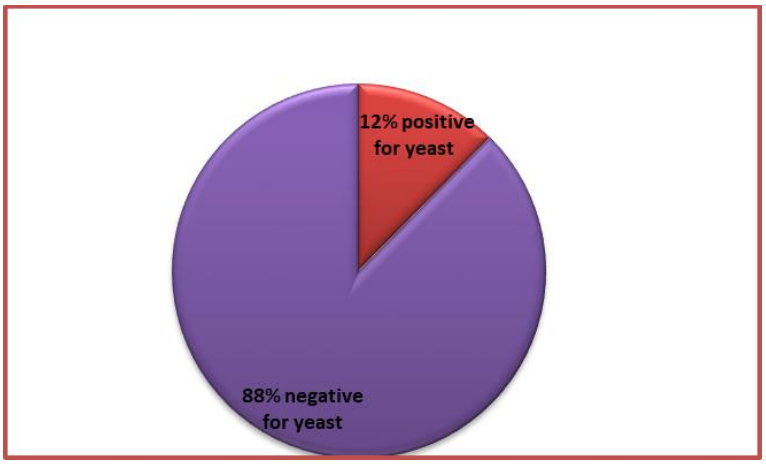

Figure: (4) Show Presence of yeast: 12% positive of yeast and 88% negative of yeast.

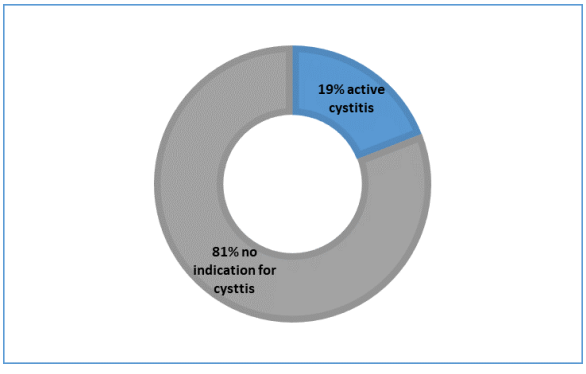

Figure: (5) show 19% active cystitis and 81% no indication for cystitis

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Table: (1) presence and absence of cast among the study Population: Cast positive in (5) sample and negative in (84) samples

Urine cast					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	5	3.4	5.6	5.6
	Negative	84	56.4	94.4	100.0
	Total	89	59.7	100.0	

Table: (2) presence and absence of urothelial cell in smears. Urothelial cell positive in (9) samples and negative in (80) samples

Urothelial cell					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Present	9	6.0	10.1	10.1
	Absent	80	53.7	89.9	100.0
	Total	89	59.7	100.0	

Table :(3) presence and absence of intermediated epithelial cell in smears intermediated epithelial cells positive in (8) samples and negative in (81) samples

Intermediated epithelial cell					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Present	8	5.4	9.0	9.0
	Absent	81	54.4	91.0	100.0
	Total	89	59.7	100.0	

Table :(4) presence and absence of corpera amylacea in smears positive in (5) samples and negative in 84 samples

Corpera amylacea					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Present	5	3.4	5.6	5.6
	Absent	84	56.4	94.4	100.0
	Total	89	59.7	100.0	

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Table :(5) presence and absence of Condyloma acuinatum in smears. Condyloma acuinatum positive in (1) sample and negative in (88) samples

(HPV infection) Condyloma acuinatum					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	1	.7	1.1	1.1
	Negative	88	59.1	98.9	100.0
	Total	89	59.7	100.0	

Table :(6) presence and absence of hematuria among the study population: Hematuria positive in (1) sample and negative in (88) samples

Hematuria					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	1	.7	1.1	1.1
	Negative	88	59.1	98.9	100.0
	Total	89	59.7	100.0	

DISCUSSION

Among 89 patients (88 males and 1 female) represent study group 36/89 (40%) are workers and 53/89 (60%) are students(figure -1), these students were not school students but were (Massed) students an immigrants whose attain to Mosque, study and to know by heart Holy Qur'an.

Those students in most instances have poor hygiene and in regular contact with agriculture water, which is the main source of urinary Schistosomiasis for swimming during the long summer season.

The age range from (8-80) years with median age (24) years. Age (11-20) is most affected group (figure -2).

Microscopic examination of stained smears shows urine crystals in 24/89 (27%), while 65/89 (73%) of samples were negative of crystals (figure -3). 11/89 (12%) of samles contains yeast cells and 78/89 (88%) were negative of yeast (figure -4). 5/89 (6%) contains urine cast and 84/89 (94%) were negative for cast, positive cases as follow: 3/5 hyaline cast and 2/5 cellular cast (table -1).

Cytologic evaluation of the smears show in mostly hypocellular smears, most slides contains from (3-8) cells .cellularity according to the type of cells as follow:

Normal urothelial cell present in 9/89 samples (10%) as isolated single cells with round nuclei, dense cytoplasm stained blue/green, with well define borders (table -2). Intermediated epithelial cells present in 9/89 (9%) (Table -3), they appear as small cuboidal cells stain

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blue/green with small nucleus similar to parabadal cells. Corpera amylacea present in 5/89 samples (6%) (Table -4). Condyloma acuinatum which is indication for human papilloma virus infection, present in 1/89 sample (1%) (Table -5). Cells show Koliocytosis similar to that of cervical infection.

Only one patient has hematuria (1%) (table -6), although the smear is tested twice in two prepared smears but Schistosoma ova were not detected instead there is also inflammatory cells, bacteria and cellular debris which indicate active cystitis (figure -5). In addition to this sample another (16) samples to give total 17/89 that account (19%).

CONCLUSION AND RECOMMENDATION

- In conclusion Pap stain is satisfactory to screen urine cytology can provide excellent details for urine contents.
- Special attention should paid by health care providers to school age children more precisely (Massed) students and all people at risk of S. hematobium infection.
- Patients who's already diagnosed should be advice to attain for regular urine test in order to detect any abnormality early

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