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Full Length Research Paper

Evaluation of the Performance of Preservation Methods in the Detection of *Schistosoma Haematobium* Ova in Urine Samples

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Abstract

The quality of formalin, boric acid, domestic bleach and carbol fuchsin were investigated as preservatives of Schistosoma haematobium ova for three weeks. Urine specimens obtained from patients included in a survey conducted in the Maio area in Khartoum the urine specimen was divided into 4 aliquots within 12h of the collection into the four preservatives mentioned above and were processed after one week, two week and three week of wet preparation of 14 positive specimens (a total of 217 processed samples) after concentration by centrifugation. We found that all fixatives give good morphological characteristics for S. Haematobium with some morphological changes, and crystal formation with formalin, boric acid and domestic bleach which appear after the first week. Formalin is aldehyde base fixative, domestic bleach is oxidizing agents based fixative, both have action of cross linking in preservation of samples which may cause these morphological changes. Carbol fuchsin gave the same morphological appearance during the three weeks. According to this result, we conclude that non-cross linking alcohol based fixatives such as carbol fuchsin gave good preservation for the morphology of S. Haematobium ova as the morphological characters did not change during preservation time.

Keywords: Urine, infection, Parasitology, eggs.

INTRODUCTION

Schistosomiasis is a parasitic disease caused by blood flukes (trematodes) of the genus Schistosoma. After malaria and intestinal helminthiasis, Schistosomiasis the third most devastating tropical disease in the world, being a major source of morbidity and mortality in developing countries in Africa, South America, the Middle East, and Asia. Urinary Schistosomiasis is a human disease condition, which is caused by infection of the trematode Schistosoma heamatobium. The parasite is found in the venous plexus draining the urinary bladder of humans. During infection, the parasites deposit terminal spines eggs, which clog the venous plexus, impeding blood flow. This bursts the veins, allowing blood and eggs to enter the urinary bladder, resulting in the characteristic symptom of blood in urine or Haematuria. In sub-Saharan Africa alone it is estimated that 70 million individuals experience heamaturia, 32million with difficulty in urinating (dysuria), 18 million with bladder-wall pathology, and 10 million with major hydronephrosis from infection caused by Schistosoma haematobium. Mortality rate due to non-functioning kidney (from S. Haematobium) and haematemesis has been put at 150,000 per year. Definitive diagnosis of urinary schistosomiasis is dependent on the demonstration of parasite eggs in urine, and more recently the detection of circulating antigens, Verani and Shane, 2011. Two new techniques for the quantitative determination of Schistosoma

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haematobium egg densities in urine samples, the first using sedimentation and the second filtration.

The first indication of Schistosoma haematobium infection is haematuria which can be detected either by a chemical test of microscopically. A heavy infection with Schistosoma may lead to gross haematuria which is seen visually. A special care is needed for the collection of urine for suspected Schistosomiasis because the number of ova excreted in urine varies throughout the day. It is highest in the terminal portion of the urine between 10.00am to 2.00pm. The last few drops of urine contain the maximum number of eggs.

Therefore, the specimen should be collected between these times and should be terminal urine at least 10ml in volume. If it is not possible to examine the fresh specimen within one hour of collection, should be preserved with fixative. If not preserved, the eggs may hatch to release miracidia. There are different fixatives use for the preservation of Schistosoma haematobium ova. In this study, we use formalin, boric acid, domestic bleach, carbol fuchsin to determine their efficacy to preserve Schistosoma haematobium ova and if there are changes of ova morphology during time of three weeks.

Formalin is a traditional fixative, have been widely used in most public, private, and commercial laboratories for many years. It is still considered the “gold standard” in Parasitology because they allow excellent long-term preservation of intestinal parasites, Garcia and Shimizu, 1976. Formalin is considered an all-purpose fixative used to preserve helminth eggs, larvae, and protozoan cysts. The routinely used fixative is 10% formalin, which is 3.7% formaldehyde in water with 1% methanol the commercial formalin is a 2-phase fixative, with an initial alcohol fixation phase, followed by a cross-linking phase mediated by aldehyde, Fox *et al.,* 1985. Boric acid was first prepared by Wilhelm Homberg (1652–1715) from borax, by the action of mineral acids. Boric acid is known urine preservative. The exact mechanism of action of boric acid is unknown; generally cytotoxic to all cells. Carbol fuchsin is known as stain, but may use as a fixative because it is alcohol base. Domestic bleach also may use as fixative. It contains oxidizing agents.

MATERIALS AND METHODS

From survey was administered in the Maio area in Khartoum- Sudan, urine sample collected from 217 patients to check the infection with urinary schistosomiasis. All patients were children. We ask him to make exercise before collection. Urine collected in clean, dry, screw caps containers. The concentration sedimentation technique applied to all samples followed by microscopic examination of urine sediments. *Schistosoma haematobium* ova detected in 14 urine samples.

Positive samples divided into four sets of containers contain fixatives, formalin (10% concentration) Boric acid (commercial boric acid powder), domestic bleach (CLOROX) and carbol fuchsin (1%).

Carbol fuchsin (1%) was prepared from 10g of basic fuchsin (Hi-Media) dissolved in 100ml of methanol and 50ml of melted phenol in a flask maintained at 60°C in a water bath. This solution was made up to 1,000ml with distilled water. Carbol fuchsin (0.3%) was prepared from 33ml of the above solution diluted to 100ml with distilled water before use. Sulfuric acid (25%) was prepared from 250ml of concentrated sulfuric acid slowly added to 750ml of distilled water. Methylene blue (0.1%) was prepared from 1g of methylene blue dissolved in 1,000ml of distilled water, Grizzle *et al.,* 2008. 0.1ml from each fixative wad added to each 10ml of urine (0.1g of boric acid). After that the sample stored to be examined for Schistosoma haematobium egg morphology after 1 week, 2 and 3 week. Each week we make a wet preparation of urine samples after concentration by centrifugation. (3000rpm for 5 minutes).

DISCUSSION

It is to be mentioned that, there are no previous studies conducted on the preservation of *Schistosoma haematobiom* ova so as to compare with our results. In general, there are 4 major groups of fixatives, namely the aldehydes, oxidizing agents, alcohol based fixatives and the metallic group of fixatives. The aldehydes based fixatives (such as formalin) and oxidizing agents based fixatives (such as domestic bleach) act by cross-linking proteins. Alcohol based fixatives (such as carbol fuchsin) are protein-denaturing agents, Fox *et al.,* 1985. All fixatives were good for the preservation of *Schistosoma haematobium* eggs in a week (1).

But in week 2 and 3 week observe morphological changes were observed with formalin, domestic bleach and boric acid fixatives which not occur with the carbol fuchsin preserved urine sample. This mean alcohol based fixatives such as carbol fuchsin which act by protein denaturation give better results than other cross linking fixatives such as formalin and domestic bleach, this is similar to a study conducted by van Essen HF, which found that non-cross linking alcohol based fixative give better results than other types of fixatives which do not change with time.

In this study, we observe crystal formation in formalin preserved urine samples appear in a week (3) this is similar to previous study made by Rooban Thavarajah, Vidya Kazhiyur Mudimbaimannar (2012) which shown that, formalin, when stored for longer periods, gets oxidized to form formic acid. Also reacts with blood to form a birefringent crystal called formalin pigments. In this study, we found that significant morphological changes occur in a week (3) formalin preserved urine samples which I think to be occurring due to the small

RESULTS

Table 1. Effects of preservative on morphological changes, background and internal structures of the ova in Week (1)

Fixative	Ova outline	Meracidium	Spine	Background (crystals
%10 formalin	Normal	well preserved	well preserved	-
Boric acid	Normal	well preserved	well preserved	-
Carbol fuchsin	Normal	well preserved	well preserved	-
Domestic bleach	Normal	well preserved	well preserved	+

Table 2. Effects of the preservative on morphological characters, background and internal structures of the ova in week 2

Fixative	Ova outline	Meracidium	Spine	Background(crystals)
10% formalin	Normal (80 %)	Abnormal (5%)	Some broken(5 %)	-
Boric acid	Normal (96 %)	Abnormal(4%)	Some broken (4 %)	-
Carbol fuchsin	Normal (100 %)	Well preserved (100%)	Well preserved (100 %)	-
Domestic bleach	Normal (100 %)	Well preserved) (100%)	Well preserved (98 %)	++

Table 3. Effects of preservative on morphological characters, background and internal structures of the ova in Week (3)

Fixative	Ova outline	Meracidium	Spine	Background (crystals)
10% formalin	Normal (20%), Abnormal (80%)	Normal (80 %)	Normal (80 %)	+
Boric acid	Normal 90 %), Abnormal (10 %)	Normal (10 %)	Normal (10 %)	-
Carbol fuchsin	Normal (100%),	Well preserved	Well preserved	-
Domestic bleach	Normal (80%), Abnormal (20%)	Normal (10 %)	Normal (15 %)	+++

amount of formalin used. Many studies say that the formalin must used by equal volume with the sample. Boric acid is known urine preservative. The exact mechanism of action of boric acid is unknown; generally cytotoxic to all cells. It provides good fixation for Schistosoma haematobium ova during the three weeks with few morphological changes occur during the second and the third week. In my opinion, this may occur due to the acidity of the fixative. The Clorox, which has been used in this study as bleach, it gives good egg morphology in the three weeks with some morphological changes also due to cross linking formation like formalin. It has a crystals appear in each examination, which may cover some eggs. The action of the oxidizing agent may responsible for the formation of the crystals in my opinion.

CONCLUSION

Through our research, we conclude that formalin, boric acid, carbol fuchsin and domestic bleach give a good morphological appearance for S. Haematobium ova. But, there are some morphological changes were observed with formalin, domestic bleach and boric acid with increasing of fixation time due to the cross linking action of these fixatives and the acidity of boric acid. On the other hand, Schistosoma haematobium ova fixed in carbol fuchsin which is a non-cross linking alcohol based fixatives give excellent egg morphology which do not change during the time. Consequently, we recommended using of carbol fuchsin for transporting and preservation of urine for S, haematobium ova, and using the correct amount of fixatives.

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