Role of Trichrome Staining Techniques in the diagnosis of Intestinal Parasitic Infections

S. Shoaib (Departments of Pathology, Dow Medical College and Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi.)
A. Hafiz (Departments of Pathology, Microbiology Jinnah Postgraduate Medical Centre, Karachi.)
S. Tauheed (Departments of Pathology, Physiology, Jinnah Postgraduate Medical Centre, Karachi.)

Abstract

Objecfive: To compare the diagnostic yield of saline and iodine preparation with trichmme staining of fresh sample of stool for diagnosis of parasitic infection.
Method: Stools were examined in saline and iodine preparation and tnchrome stained smears prepared according to the method recommended by WHO in oil immersion.
Results: Out of 400 specimens 215 were positive for parasitic infection and of these 81 were diagnosed exclusively on tnchmme stained samples.
Conclusion: Because of its better diagnostic field tnchmme staining technique should be included in the routine stool examination (JPMA 52:152;2002).

Introduction

Parasitic infections persist as a global health problem. Among these, intestinal parasitic infections are the most prevalent in man[1]. Identification of intestinal parasites rests upon demonstration of cysts, ova or trophozoites in stool samples[2] and examination of wet mounts of stool samples in saline and iodine is the conventional routinely used method for this purpose.
Permanent staining of faecal smears by trichrome technique has been used by a few scientists for the detection of parasites in the past and it was found to be highly sensitive. This technique was first used by Pollack and Mars in 1944 for vaginal smears[3]. Gomori, in 1950 reported that by combining trichrome ingredients in a single solution, an excellent polychromatic differentiation can be obtained in tissue smears[4] and currently this technique is used for tissue differentiation in the field of histopathology. Wheatly, in 1951, applied this technique for the demonstration of Entamoeba histolytica cysts and trophozoites in a fatal case of amebiasis[5]. Since these early studies, trichrome staining method has been known to be a sensitive diagnostic tool for the detection of intestinal parasites also but unfortunately it has not gained its due importance in many countries including Pakistan.
The aim of this study was to highlight the usefulness of trichrome staining method in faecal smears for the diagnosis of intestinal parasitic infections.

Materials and Method

A total of 400 cases presenting with acute, intermittent or persistent diarrhoea with anorexia, anaemia, abdominal pain and passage of worms or allergic reactions were included in this study. Stool samples were collected in clean, wide mouth, dry bottles with lids. With the help of applicator stick, a small amount of faecal sample was applied in the centre of a clean glass slide and a thin, uniform smear was made. If the stool was hard, a few drops of saline were added to soften it. Schaudinn's fixative was

heated to 60°C. The smears were flooded with the fixative. The use of preheated fixative reduces the fixation time from 30 minutes to 10 minutes, which helps in providing results in a shorter duration[6]. The smears were processed according to the method recommended by WHO[7]. The smear was covered with iodine alcohol solution for one minute. It was washed and dipped in ethanol (70%) for one minute then placed in a Coplin jar containing trichrome stain for 5 minutes and destained with acid-alcohol destaining solution for a few seconds. It was then placed in ethanol (95%), absolute alcohol and xylene for one minute each respectively.
In order to compare the results, each sample was also examined by preparing wet mounts in saline and iodine. The trichrome stained smears were examined in oil immersion.

Results

In the 400 stool samples examined for parasites 215 were found to be positive. By using trichrome technique, parasites were detected in all 215 positive cases while with the use of wet mounts in saline and iodine parasites were identified in only 134 (62.3%) apecimens. It was observed that permanently stained smears with trichrome proved to be very effective for the detection of parasites especially protozoa in their vegetative and cystic forms (Table).

Table. Identification of various parasites wet mounts and trichrome with comparison of both techniques.

Parasites	Number of cases identified	Identification by trichrome		Identification by wet mount	
		No.	%	No.	%
Giardia intestinalis	70	70 C=53	100 V=17	40 C=31	47 V=9
Entamoeba histolytica	67	67 C=46	100 V=21	32 C=20	47 V=12
Entamoeba coli	20	20 C=20	100	20	100
Endolimax nana	5	5 C=5	100	-	-
Iodamoeba buetschlii	3	3 C=3	100	-	-
Dientamoeba fragilis	8	8*	100 V=8	-	-
Blastocystis hominis	21	21	100	21*	100
Ascaris	30	30	100	30*	100
Trichuris trichura	22	22	100	22*	100
Hymenolepis nana	8	8	100	8*	100
Enterobius vermicularis	3	3	100	3*	100
S. Stercoralis	1	1	100	1*	100
Taenia species	1	1	100	1*	100

C= Cystic forms
V= Vegetative forms

The internal elements that distinguish trophozoites from cysts and other structures were clearly visualized and the morphology of parasite trophozoite and cystic stages was enhanced. The cytoplasm of trophozoites and cysts appeared dark green while the chromatin, ingested RBC and bacteria inside trophozoites appeared reddish purple, thus the cell components of the parasites became prominently visible by taking the stain against the pale background and it was observed that 81 samples were

diagnosed exclusively by this method. Trichrome staining method was found to be more sensitive for the diagnosis of protozoa than helminths (Table).

Discussion

Intestinal parasitic infections present with diarrhoea which may be acute or intermittent in nature. The clinical features are not adequate as a diagnostic guide to the responsible agents involved[8]. Correct identification of parasites is essential before starting the treatment with anthelmentics as these drugs are mostly active against specific groups of parasites and some are toxic also[9]. In all the cases, the defmite diagnosis rests on examination of stool samples. It has been noted that examination of direct wet mounts of stool samples in saline and iodine as is done routinely is not adequate especially for protozoa which are more difficult to detect due to their smaller size and resemblance with other structures such as food particles, air bubbles and vegetable or fat cells. Another disadvantage of wet mount technique is that the slide preparations are temporary, a short time interval is available for the test and as the slides can not be kept for a long time therefore they are not available for future reference. As a result, the diagnosis relies on the experience of the technician only.

Permanently stained smears of faecal samples, by trichrome provide a better means for the detection and diagnosis of parasitic infections as compared to the conventional wet mounts. Schaudinn's fixative, which is used in this technique, preserves the morphology of the vegetative forms as well as cysts of protozoa. Chromotropc 2R, which is an ingredient of trichrome stain, has a strong affinity with the chromatin. The cytoplasm of vegetative forms appears dark green while the nuclear chromatin appears dark red in colour. The morphological features of vegetative forms become prominent against the pale background thus making the visibility of protozoa more prominent and increasing the sensitivity of this method[9,10]. Some protozoa such as Dientamoeba fragilis which exist in vegetative form only can be detected exclusively by this method[6,9] as was also observed in this study.

Trichrome staining method was compared with other staining methods like Heidenham's iron haematoxyline, Faust's haematoxyline and Merthiolate iodine formaldehyde by Vinayak and Prakash in 1967, [11] and it was found to be 95% sensitive as compared to 70% sensitivity of iron haematoxylene, 65% for Faust's haematoxylene and 68% for Merthiolate iodine formaldehyde technique.

In the present study out of 400 stool samples, 215 were found to be positive for intestinal parasites and 81(37.6%) samples were diagnosed exclusively by trichrome method. The diagnosis in these 81 cases would have been missed if only wet mount examination was done. It was observed that a combination of techniques can yield a greater number of positive cases than does any one technique alone.

In this study a review of the simple, fundamental methods for examination of stool samples was carried out by comparing the routinely used wet mounts in saline and iodine and trichrome technique. It was noted that although permanent staining by trichrome method requires some knowledge and experience like any other laboratory technique but it is more reliable, sensitive and has the advantage of providing permanent slides which can be referred to the pathologist for confirmation instead of relying on the report by he technical staff. This method has been recommended by WHO and the American Society of Parasitologists[12] but still has not gained its due importance. It is suggested that trichrome method should be introduced and applied for the diagnosis of parasitic infections in order to avoid incorrect or missed diagnosis. This method can be used in combination with direct wet mounts to confirm specific identification of suspected organisms in parasitic infections.

References

1.Chatterjee KD, Parasitology and helminthology. Srec Saraswaty Press Ltd. Calcutta 1978,pp. 207.11.

2.Mims CA, Playfair ill, Toill IMI, et al. Medical Microbiology. Fiona Foley Mosby-year Book Ltd., 3rd edition, London, l993;25:25-30.
3.Pollack OJ, MarsT. A rapid trichrome stain. Arch. Pathol., 1944-37:294.
4.Gomori G. A rapid triehrome stain. Am. J. Clin. Palhol., 1950; 20:661-64.
5.Wheatley WB. A rapid staining procedure for intestinal amoebae and flagellates. Am. J. Clin. Pathol., 1952; 21:990-91.
6.Baron EJ, Peterson LR. Finegold SM. Bailey and Scon's diagnostic Microbiology, 9th (ed). Louis: Mosby, New York, 1998, pp. 781.88.
7.World Health organization. Laboratory methods in Medical Parasitology, Geneva: WHO, 1991,pp. 9-24.
8.Association of Centre for Disease Control. Atlanta. Intestinal parasites in United States. J. Am. Med. Assoc, 1978; 239: 2756.
9.Katzung BG. Anthelmintic drugs. Basic and clinical Pharmacology. New York: Lange Medical Books, 1992; 5:748-66.
10.Eslevez EG, Judith AL, Examination of preserved stool specimen for parasites. Lack of value of direct wet mount. J, Clin. Microhiol. 1985; 6: 666-67.
11.Vinayak VK, Prakash 0. Comparative evaluation of some staining techniquc.s for intestinal protozoa instool specimen. indian J. Med. Res., 1967; 5511:674 77.
12.Abadie SH, Beaver TC, Brukner D. et al. Recommended procedures for the examination of clinical specimens submitted for the diagnosis of parasitic infection, Am. J. Med. Technol., 1978; 10: 1101-6.