ORIGINAL RESEARCH PAPER

Volume-7 | Issue-3 | March-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

IS IMMUNOHISTOCHEMISTRY BEST CONFIRMATORY TEST IN DIAGNOSIS OF COMPLICATED CASES OF AMOEBIC COLITIS?

Pathology

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ABSTRACT

Subtitle: Immunohistochemistry in amoebic colitis

Background: Amoebic colitis is a common large intestinal infection caused by anaerobic parasite E. Histolytica. This study was done to compare efficacy of Periodic acid schiff's (PAS) and immunohistochemical (IHC) marker in diagnosis of amoebic colitis.

Material & methods: 30 cases of amoebic colitis reported on Haematoxylin & Eosin (H&E) were studied. Average number of trophozoites recognized in 10 high power fields on PAS and IHC were compared.

Observation & results:

1. Out of 30 cases of amoebic colitis reported on routine H&E, 23 cases recognized with trophozoites on PAS while 20 out of 30 cases were positive for trophozoites on IHC.
2. Number of trophozoites identified was 5 times on IHC and 2 times on PAS in comparison to H&E.

Conclusion: IHC provides accurate diagnosis of amoebic colitis. It gives intense brown colour which makes trophozoites identification more easier. With the use of IHC false positive diagnosis of amoebiasis can also be curtailed.

KEYWORDS

Amoebic colitis, Immunohistochemistry, Masson's trichrome, Periodic acid schiff's.

INTRODUCTION

Entamoeba histolytica (E. histolytica) is a common enteric parasite that causes about 500 million infestations and deaths over 100,000 worldwide every year [1].

Amoebic colitis (AC) is the third leading cause of death among parasitic infestations after malaria and schistosomiasis [1]. Majority of infested patients remain asymptomatic and only 10% may develop diarrhea, dysentery, invasive colitis, liver abscess, multiple large intestinal perforations or peritonitis [2]. In literature, we found very few studies on importance of Immunohistochemistry (IHC) marker over Periodic acid schiff's (PAS) and Haematoxylin & Eosin (H&E) stain in diagnosing amoebiasis ex vivo but Literature didn't reveal any study in which IHC marker has been used on paraffin sections of amoebic colitis in human tissue. It's a devastating disease and should be diagnosed as accurately as possible.

MATERIAL AND METHODS

Present study was conducted in the Department of Pathology, University College of Medical Sciences & Guru Teg Bahadur Hospital, Delhi. Histopathology numbers of already diagnosed 30 cases of amoebic colitis on H&E between 2011 and 2014 were retrieved. Paraffin blocks of representative sections of these cases were retrieved. Two sections of each block were taken and stained with PAS and IHC for E. histolytica (Thermo Fisher- E. histolytica polyclonal antibody, D42A).Study was conducted after obtaining ethical clearance from the Institutional Ethics Committee.

Procedure for each stain is as follows:

PAS: Staining was done according to standard protocol [3]. Sections deparaffinized and hydrated to water then oxidized in 0.5% periodic acid solution for 5 minutes and rinsed in distilled water. Schiff's reagent was placed for 15 minutes and then washed in lukewarm tap water for 5 minutes. Counterstaining was done by using alum haematoxylin for 1 minute and washed in tap water for 5 minutes, dehydrated, cleared and mounted with DPX.

Labelled Streptavidin biotin (LSAB) method of E. Histolytica Immunostaining

Staining was performed on processed tissue sections with some modifications of the standard protocol as described by Bancroft and Gamble [3]. Immunostaining for E. Histolytica was done to confirm presence of E. histolytica trophozoites. Three-four micron thick sections were taken on lysinated slides, deparaffinized with xylene and rehydrated with graded alcohol and washed with phosphate buffer saline (PBS) at pH 7.2. Slides were transferred to staining dish containing antigen retrieval solution in the microwave retrieval chamber for 15-20 minutes and washed with PBS at pH 7.2. Then endogenous peroxide blocking was done by hydrogen peroxide (4%) for 5-10 minutes. Slides were incubated with the primary antibody against E. histolytica for 120 minutes and washed with PBS at pH 7.2 for 10 minutes. Thereafter, slides were incubated with preformed avidin-biotinylated peroxide antibody complex for 20 minutes.

These were then, incubated with diaminobenzidine (DAB) solution for 10 minutes and rinsed with PBS at pH 7.2 and transferred to running tap water. Counterstaining was done by haematoxylin followed by rehydration and clearing. Mounting was done by using DPX.

Trophozoites of E. Histolytica were counted in 10 high power fields (HPF) in the same area of the section in both stains. Efficacy of stains in terms of median number of trophozoites recognized per 10HPF were counted and compared.

OBSERVATION& RESULTS

On H&E, trophozoites were recognized as round to oval structures ranging in size from 20-90µ (of size of small lymphocyte) having pyknotic nuclei, abundant vacuolated eosinophilic cytoplasm & variable number of ingested intact and/or fragmented red blood cells in the cytoplasm. Organisms were present in the necrotic background with presence of inflammatory cells including neutrophils, lymphocytes, macrophages & few plasma cells. Trophozoites were found, not only at the ulcer edges but also in the entire thickness of intestinal wall. (Figure 1)

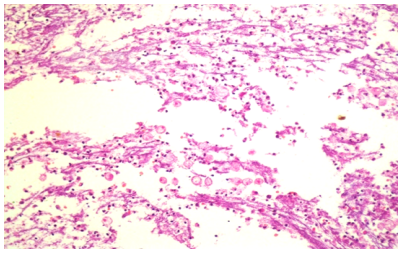

Figure 1; Section of colon shows pink coloured round to oval amoebic trophozoites lying in a necrotic background (H&EX200, original magnification).

On PAS, trophozoites were identified as round to ovoid bodies with magenta colour cytoplasm. (Figure 2)

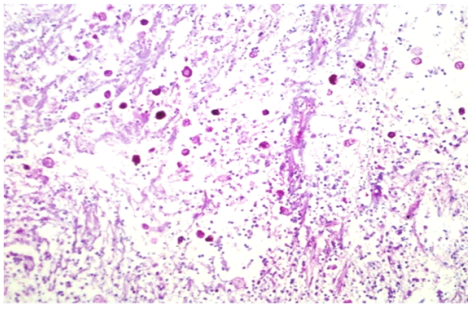

Figure 2; Shows trophozoites stained magenta in colour (PASX200, original magnification).

Immunostaining for E. Histolytica was done and trophozoites appeared as intense brown coloured, round to oval to fragmented structures in a pale blue background. (Figure 3)

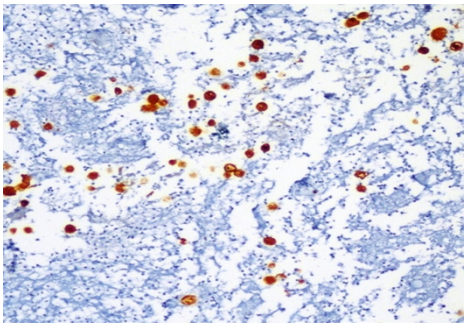

Figure 3; Shows intense brown colour stained trophozoites of E. histolytica, present in well contrast blue background. (E. histolytica antibody IHCX200, original magnification).

PAS and IHC revealed trophozoites in 26 and 20 out of 30 cases diagnosed as amoebic colitis on routine H&E stain. 10 cases reported as amoebic colitis on H&E stain, turned out negative for E. histolytica on IHC. Trophozoite count was highest per 10 HPF on IHC as it was 5 times of H&E and 3 times of PAS stain.

DISCUSSION

Amoebiasis occurs worldwide; but its prevalence is disproportionately high in developing countries because of poor socioeconomic status. In some areas the overall prevalence of amoebic infection is as high as 50 percent and according to one study conducted in Calcutta, prevalence rate is 3.6% in India [4, 5]. Intestinal perforation by amoebic colitis remains a common and an important cause for emergency colectomy [6, 7].

Many serological techniques have been used for detection of amoebic infection so far [8, 9, 10].

But they are not much informative as antibodies remain in blood for longer period even after recovery.

In present study, we have done PAS and IHC for E. histolytica in all the cases. PAS was positive in 23 cases and negative in seven cases. Seven cases negative on PAS could be explained due to less number of trophozoites, or fragmented trophozoites or cases wrongly diagnosed due to confusion between macrophages and trophozoites on H&E stain. Trophozoites of E. histolytica stained magenta in colour on PAS. PAS stains the glycogen containing structures present within the cytoplasm of the cells therefore sometimes macrophages also take the stain. Hence in view of the similarities in morphology and size, it often becomes difficult to differentiate trophozoites from macrophages in PAS stain and create dubious situation in making diagnosis [4].

Ning et al in their study found that IHC is the best marker in diagnosis of amoebic liver abscess over routinely used stains. In our study IHC was positive in 20 out of 30 cases of amoebic colitis while 10 cases were negative. It could be due to ulcer caused by some other strains of amoeba besides E. histolytica as IHC marker was specific for E. histolytica or cases were misdiagnosed on H&E. It was easy to find out trophozoites on IHC owing to their intense brown colour against well contrast background. Therefore number of trophozoites per 10HPF was also high on IHC; approximately 5 times of H&E.

CONCLUSION

Amongst the special stains PAS is a good supplementary stain for diagnosing amoebiasis. But in certain cases it doesn't provide valuable information due to confusion with macrophages. IHC proved to be a sensitive marker in recognization of trophozoites in cases where their number was less. IHC was found to be superior especially in those cases where fragmented trophozoites were seen or were confused with macrophages. Negative staining for IHC in 10 cases in our study calls for the immunostaining for other strains like E. Dispar and E. Moshkovskii.

ACKNOWLEDEMENT

This study would not have been completed without immense support and apt guidance given by my best friend Mr. Kaustav Shrivastav.

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