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METHYL GREEN PYRONIN: A PROGRESSIVE NUCLEAR STAIN SUBSTITUTE FOR HAEMATOXYLIN AND EOSIN STAINING TECHNIQUE IN THE DEMONSTRATION OF INVASIVE DUCTAL CARCINOMA

ALAEZI CM^{1*}, ADISA JO², OKOROCHI EC³ and OKECHI OO⁴

- 1- Department of Medical Laboratory Science, Imo State University, Owerri, Imo State
- 2- Department of Histopathology/Pathology, Jos University Teaching Hospital, Plateau State
- 3- Department of Chemical Pathology, Federal Medical Centre, Owerri, Imo State
- 4- Department of Morbid Anatomy, Abia State University Uturu Abia State

Corresponding Author: ALAEZI CM

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ABSTRACT

Objective: This study aims at comparing two histological techniques for the demonstration of invasive ductal carcinoma. **Method:** Haematoxylin and Eosin technique and methyl green pyronin staining techniques were exploited. Methyl green pyronin (MGP) stain was prepared at various concentrations, pH and applied for different lengths of time on 5 micrometer thick sections of the breast tissue diagnosed to have invasive ductal carcinoma. Haematoxylin and Eosin (H & E) stain was used as control on the same tissue. All the stained sections were examined microscopically. **Result:** The MGP stained sections showed a well demonstrated irregular duct - like spaces and long narrow cords of similar cells but poorly demonstrated malignant cells. The fibrous stroma infiltrated with lymphocytes was partially demonstrated like in H & E stained tissue. Decreasing concentration of MGP stain gave poorer demonstration of the various diagnostic features of invasive ductal carcinoma. At pH 2.0, diagnostic features of invasive ductal carcinoma were generally well demonstrated, while beyond neutral pH, all diagnostic features were poorly demonstrated. Duration of treatment did not affect the staining reaction with MGP.

Keywords: *Methyl Green Pyronin, Nuclear Stain, Haematoxylin, Eosin Staining Technique, Carcinoma.*

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INTRODUCTION

Breast cancer is the cancer that affects the breast and is the most common cancer and the second principal cause of cancer deaths in women worldwide as well as in Nigeria (Adebamowo, 2000).

The need for accurate and sensitive methods for demonstrating breast cancer for early detection can therefore not be overemphasized. It is for this reason that methyl green pyronin stain, a special stain known to demonstrate nucleic acid in the nucleus, which is vital to the diagnosis of any cancer was used to ascertain if it could act as a substitute for haematoxylin and eosin staining technique, a routine staining technique in histology. This study however aims at comparing two histological techniques for the demonstration of invasive ductal carcinoma.

MATERIALS AND METHODS

Paraffin wax embedded surgical breast tumors were obtained from the histopathology department, Jos University Teaching Hospital, Plateau State. The tumor cases selected were from the breast already confirmed to be invasive ductal carcinoma. These were sectioned at 5 micron using a rotary microtome. A control staining technique known as haematoxylin and eosin was used to assess the test stain 'methyl green pyronin'. Invasive ductal carcinoma tissue sections were taken to water, rinsed in acetate buffer (PH 4.8) and stained with the various solution of methyl green pyronin for the different experimental staining time which were within 30 minutes and 1 hour, concentration to include normal concentration and doubling concentration, pH ranging from 0.5, 2.0, 7.0 and 10.0 respectively.

RESULTS AND DISCUSSION

RESULTS

The Methyl green pyronin (MGP) stained sections at pH 2.0 (acidic pH) showed a well demonstrated irregular duct-like spaces and long narrow cords of similar cells but poorly demonstrated malignant cells (Plate 4) as compared to H & E stained section. The fibrous stroma infiltrated with lymphocytes was partially demonstrated at normal concentration as in H & E stained section (Plate 1 and Plate 2 respectively). Decreasing concentration of MGP stain gave poorer demonstration of the various diagnostic features of invasive ductal carcinoma (Plate 3). At PH 2.0, diagnostic features of invasive ductal carcinoma were generally well demonstrated (Plate 4), while beyond neutral pH, all diagnostic features were poorly demonstrated (Plate 6). Duration of treatment did not affect the staining reaction with MGP stain.

PHOTOMICROGRAPHS OF INVASIVE DUCTAL CARCINOMA

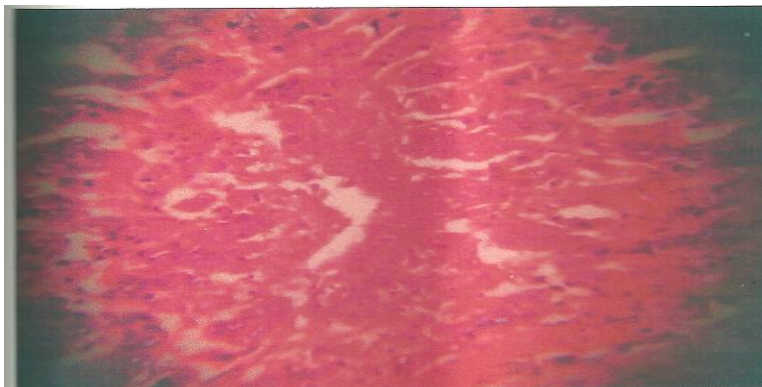


Figure 1. Haematoxylin and Eosin Stain(H&E)

Histologic Description

H&E stain showing irregular duct-like spaces, malignant cells with abundant eosinophilic cytoplasm and large moderately pleomorphic round vesicular nuclei. A few nuclei are pyknotic but most are also very pale (vacuolated) and contain one or more large nucleoli. There is considerable mitotic activity. The stroma is fibrous and infiltrated with lymphocytes and plasma cells. All these features are well demonstrated.

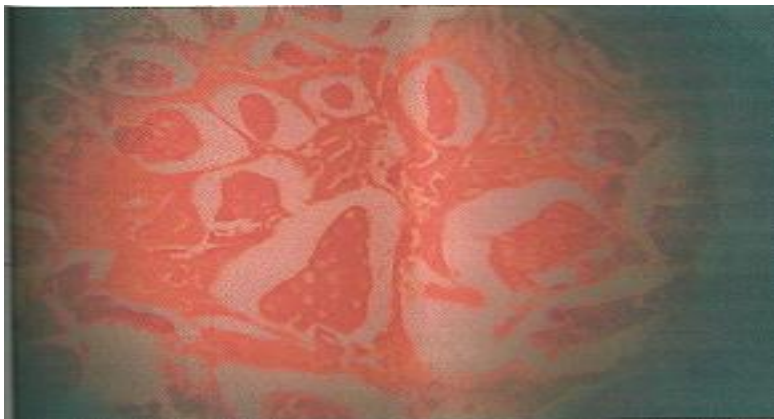


Figure 2. METHYL GREEN PYRONIN AT NORMAL CONCENTRATION

Histologic Description

MGP stain clearly showing irregular duct-like spaces and long narrow cords with similar cells showing pyroniphilic nuclei. Intense pyroniphilia of nuclei seen. Malignant cells with a few pyknotic nuclei and showing almost very pale (vacuolated) nuclei with one or more large nucleoli. Considerable mitotic activity not well demonstrated. Fibrous stroma showing infiltration of pyroniphilic lymphocytes and plasma cells, partially demonstrated.

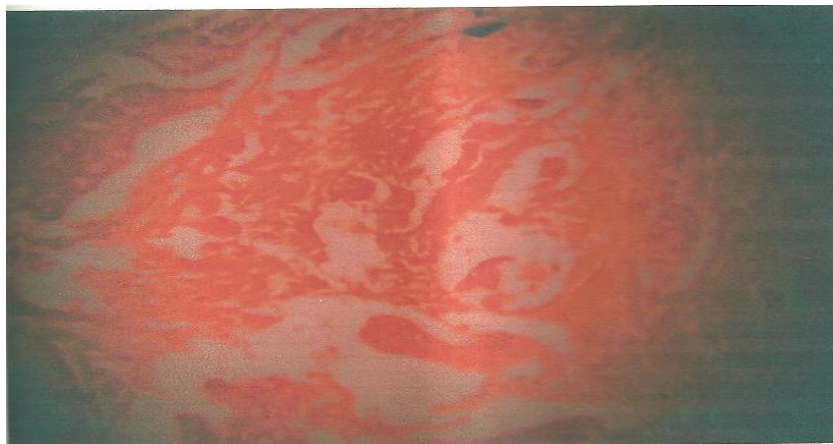


Figure 3. METHYL GREEN PYRONIN AT DOUBLING DILUTION

Histological Description

Irregular duct-like spaces, long narrow cords of similar cells showing pyroniphilic nuclei partially demonstrated. Pyroniphilia of nuclei not so intense. Malignant cells having few pyknotic nuclei and considerable mitotic activity poorly demonstrated. Infiltration of pyroniphilic lymphocytes and plasma cells into the fibrous stroma of the tissue poorly demonstrated.



Figure 4. METHYL GREEN PYRONIN AT pH 2.0

Histological Description

Irregular duct-like spaces and long narrow cords with similar cells having orange-red pyroniphilic nuclei well demonstrated. Poor demonstration of malignant cells having few pyknotic nuclei. Considerable mitotic activity and infiltration of lymphocytes and plasma cells in fibrous stroma of the tissue not clearly seen.



Figure 5. METHYL GREEN PYRONIN AT pH 7.0 (Neutral pH)

Histological Description

Loss of pyroniphilia of cells as color of stain reverts from orange-red to purple. Most diagnostic features of invasive ductal carcinoma poorly demonstrated.



Figure 6. METHYL GREEN PYRONIN STAIN AT pH 10.0

Histological Description

Loss of pyroniphilia of cells as cells revert from orange-red to purple. Most diagnostic features of invasive ductal carcinoma poorly demonstrated.

DISCUSSION

The control staining technique H&E demonstrated all the diagnostic features of invasive ductal carcinoma well. However, in comparison with methyl green pyronin (MGP) stained sections, at normal concentration of MGP stain, most diagnostic features of invasive ductal carcinoma were partially demonstrated as compared to MGP stain at doubling dilution. This observation is however in agreement with authors (Kiernan, 1990 & Lyon, 2003) which said that concentration of MGP stain could affect the staining ability of the stain.

It can further be deduced that at variation of pH, decreasing acidic pH gave an improved stainability of MGP on the diagnostic features of invasive ductal carcinoma. This finding is in agreement with what literature had, stating that the pyroniphilia of the nuclei increased with decreasing strength of the acid (Lyon, 2003).

Furthermore, beyond neutral pH of MGP stain, stainability of MGP stain is poor, which maybe as a result of the base (concentrated ammonia) used in the preparation of basic solution of MGP stain. Bases according to literature has it that they are capable of precipitating dye molecules making the solution of dye weaker. (Luna, 1998).

More so, at varying duration of treatment, there was no significant change in the staining ability of MGP stain; hence time is not a determining factor for the staining reaction of MGP stain. Finally the study carried out on MGP stain when compared with the control staining technique H&E, shows that MGP stainability is at its optimum at a given concentration and pH; hence MGP stain is concentration and pH dependent but not time – dependent. This finding is however a confirmation of literature which states, differential staining with MGP is not time-dependent but is detected by the relative concentration of MGP stain and the pH of the staining solution. (Lyon, 2003).

CONCLUSIONS

In conclusion, MGP stain is a good nuclear stain at its normal concentration which has a pH of 3.55 when compared with a control staining technique H&E.

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