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Research Article

HAIR DYE– A NOVEL INKING SUBSTITUTE IN HISTOPATHOLOGY

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A R T I C L E I N F O

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A B S T R A C T

Background: Inking agents are usually used in histopathology for marking gross specimen excision margins. Colouring the specimen with different colours adds precision to the margins. They are especially useful when tumor borders are infiltrative, irregular and excision margins are close. In this study, various coloured dyes were used and their efficacy was compared.

Aim: Aim of this study is to compare the efficacy of various commercially and easily available coloured dyes such as hair dyes, acrylic colours with the traditionally available India ink.

Materials and Methods: 30 tissue Samples were selected and grouped into 3 groups and inking was done using India ink, Hair dye and Acrylic colour for each group. All the groups were compared for their efficacy.

Results: Acrylic colour is comparatively easier for application. The drying time for india ink (3 mins ± 20 sec) was less than hair dye and acrylic colour. Out of 10 blocks made for hair dye, all displayed clear visibility on paraffin blocks (100%). 90% of those inked with acrylic colors were visible clearly and uninterruptedly under the microscope. With India ink and hair dye, 50% dehydrating agents showed contamination. However, with acrylic, clearing agent (xylene) showed contamination. There was penetration of India ink and showed mild interference with the microscopic interpretation.

Conclusion: Hair dye and acrylic colours can be used as a substitute to India ink as marking dye for histopathology.

INTRODUCTION

Coloured inks are usually used in histopathology for marking gross specimen excision margins. "Grossing" refers to examination and dissection of surgical specimens, along with preparation of sections from those tissues requiring processing. It is the initial step in surgical pathology dissection.[1] Coloring gross specimen excision margins with different colors, adds meticulousness to margin examination.[2] The purpose of inking the specimen is for determining the excision margins of the specimen, indicating specific areas of interest to the pathologists and for assisting in determining the mode of embedding.[1] Three dimensional macroscopic reconstruction of the lesion with the adjacent structures are made easier with the help of inking agents during grossing.[3] They are especially useful when tumour borders are infiltrative, irregular and excision margins are close. These inking agents help in maintaining the orientation of grossed specimens during embedding.[4] In this study, a novel inking substitute hair dye is used and compared with various coloured dyes such as india ink and acrylic colours which were generally used as a inking agent and their efficacy was compared. India ink is composed of inert carbon black with water and gelatin is widely used as a inking agent for marking resection margins.[5] Acrylic colours are composed of polyvinyl hydrochloride with coloured pigments. Hairdye contains p-phenylenediamine and phenylmethyl pyrozolone and is used to colour the hair.

Aim

To compare the efficacy of commercially and easily available colouring agent, hair dye with the traditionally used India ink and acrylic colour as marking dye in histopathology.

MATERIALS AND METHODS

The study was carried out in 30 soft tissue samples which were removed during gingivectomy and impaction procedures and these samples were divided into three groups, namely

Group A: Marking done with India Ink (10 samples)
Group B: Marking done with Hair dye; Godrej expert – Natural Black (10 samples)
Group C: Marking done with Acrylic Colour; Fevicryl - Lemon yellow and Fevicyyl - Vermilion (5 samples with yellow and 5 samples with red colour) [Figure1]
All groups were compared for their efficacy.

METHODOLOGY

The surfaces of the fixed specimen were dried using the blotting paper. In every group, one surface of the specimen was selected for inking.

Figure1 Inking agents used in the study – India ink, Acrylic colour and Hair dye

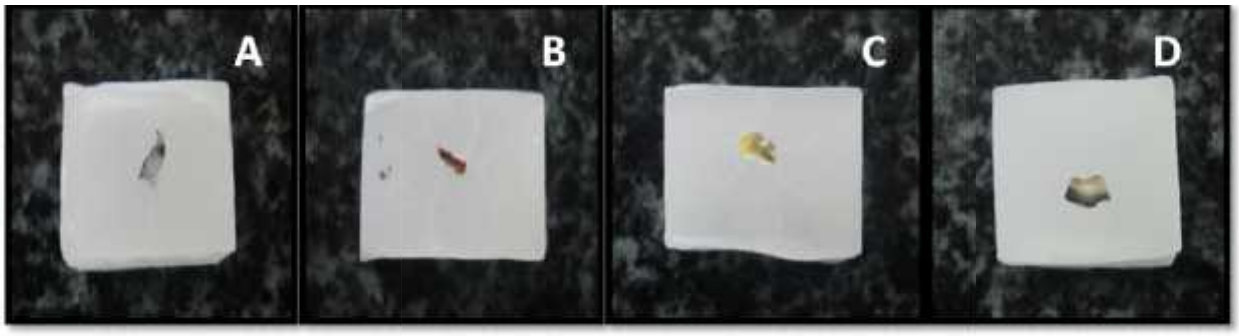

Figure 2 Macroscopic findings on Paraffin Blocks - A – India ink, B – Acrylic (Vermilion), C – Acrylic (Lemon yellow, D – Hair dye)

In most of the samples, epithelial surface of the sample was selected and painted with India ink, Hair dye and Acrylic colour (yellow and red) respectively using commercially available cotton buds. All the specimens were air dried to fix the colour and each group was processed separately by routine procedure using graded alcohol and xylene. Specimens were embedded in paraffin wax and sections of 3 µm were made using semi automated microtomes. Sections were stained with hematoxylin and eosin stain and mounted.

Following parameters were used to assess the efficacy of the inking agent: Ease of application, drying time, visibility on paraffin sections, visibility on microscopic sections and interference with cellular and nuclear details.

RESULTS

The interpretations of results of all the above mentioned parameters are given in the table 1.

Table 1

S.No	Parameter	Results
1	Ease of application	India ink (n=10) = 80% Acrylic (n=10) = 90% Hair dye (n=10) = 80%
2	Drying time	India ink (n=10) = 3.00 minutes (±20 seconds) Acrylic (n=10) = 4.30 minutes (±25 seconds) Hair dye (n=10) = 4.00 minutes (±20 seconds)
3	Visibility on paraffin blocks	India ink (n=10) = 80% Acrylic (n=10) = 80% Hair dye (n=10) = 100%
4	Visibility on microscopic examination	India ink (n=10) = 80% Acrylic (n=10) = 90% Hair dye (n=10) = 60%
5	Contamination of processing fluids	India ink – Dehydrating agent (50% alcohol) Acrylic – Clearing agent (Xylene) Hair dye - Dehydrating agent (50% alcohol)
6	Penetration of inking agent into tissue sample	India ink (n=10) - 20% Acrylic (n=10) – None Hair Dye (n=10) – None

On assessing ease of application, Acrylic colour is comparatively easier. Hair dye was found to have least drying time. In this study, macroscopic visibility on paraffin blocks was better with hair dye [Figure 2]. In some of the samples, India ink was dissolved in processing fluids and so, not found in paraffin blocks and microscopic sections too. In a few samples India ink was dissolved and it also contaminated the dehydrating agent. All samples of acrylic color (yellow) contaminated the clearing agent. On assessing the penetration of inking agent into the sample, india ink penetrated into the tissue.

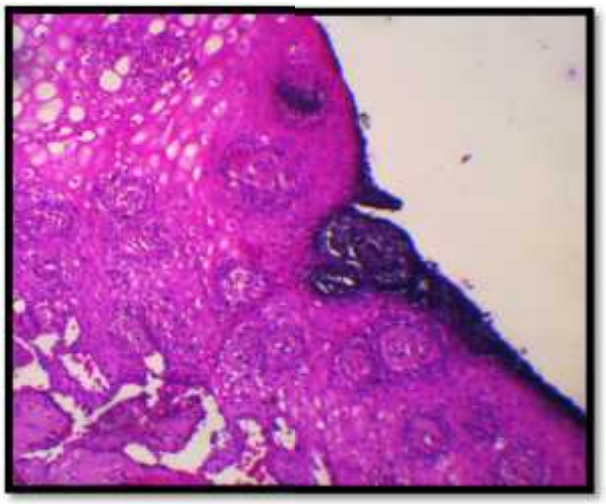

Figure3 Photomicrograph showing inking with india ink under light microscope (Hematoxylin and Eosin stain-10X)

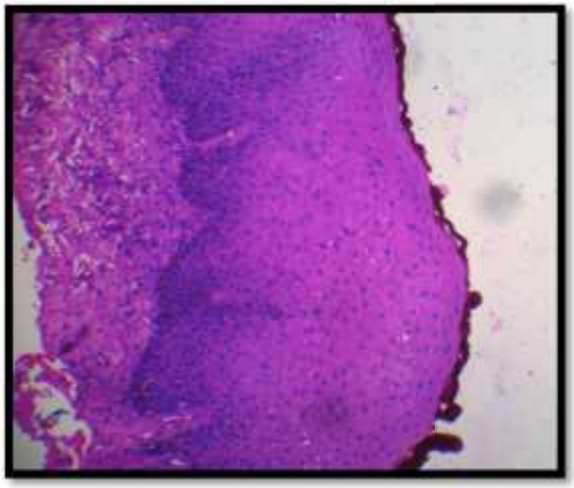

Figure 4 Photomicrograph showing inking with Acrylic (Vermilion) under light microscope (Hematoxylin and Eosin stain-10X)

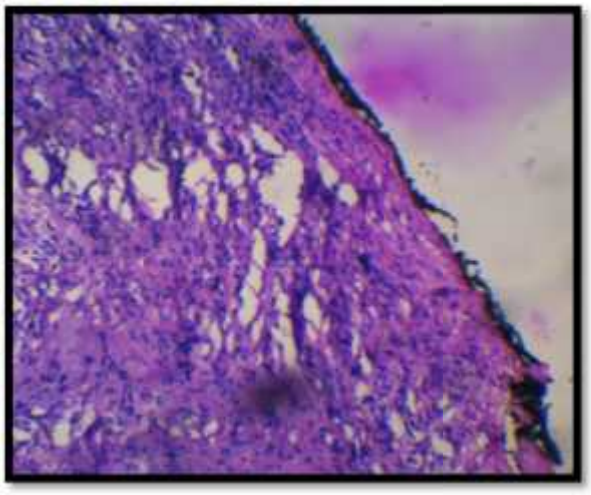

Figure 5 Photomicrograph showing inking with Acrylic (Lemon yellow) under light microscope (Hematoxylin and Eosin stain-10X)

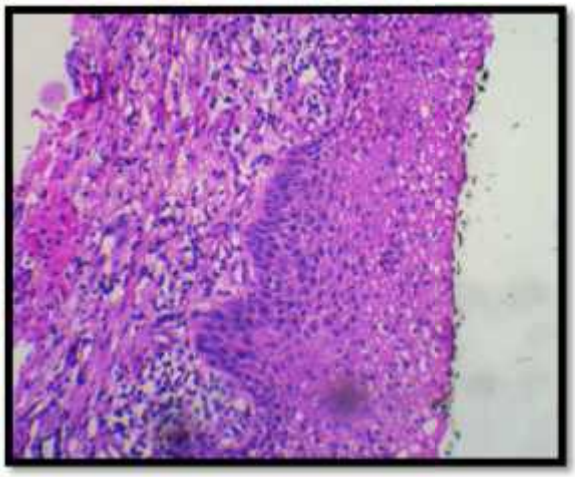

Figure 6 Photomicrograph showing inking with Hair dye under light microscope (Hematoxylin and Eosin stain-10X)

DISCUSSION

Existence of tumor cells in the resected surgical specimen margins holds significant therapeutic and prognostic implications.[6] Till now, histopathological assessment of margin status is considered the gold standard. Orientation of the excised specimen is important for both post-grossing specimen examination and microscopic identification of resected margins.[7] Inking the margins is more reliable for identification of surgical margins before and after tissue processing.[8]

India ink is composed of inert carbon black with water and gelatin, and it is widely used as a inking agent for marking resection margins. The inked specimen is treated with acetic acid, which helps in fixing the India ink, so it doesn't flow to other areas.[9] India Ink is usually used because it is able to survive tissue processing, during which the inked tissue samples are processed in alcohol and xylene and then in embedding in paraffin wax before mounting onto glass microscope slides.[2] The limitation of using india ink is that, it cannot be used to distinguish between different excision margins because of its availability in single colour.

Acrylic paints were first used in the 20th century in Mexico[3]. Acrylics are the most popularly used today because they are cost effective and they are resistant to aging. Acrylic colours are composed of polyvinyl hydrochloride with coloured pigments and available as thick emulsion[10]. However, tissue marking dyes (TMD) are available in the market but they are expensive. Even though acrylic colours are affordable, non-toxic, non-flammable and easily available in numerous colours, not all acrylic colours withstand tissue processing and fulfill the criteria of being used as surgical ink.[3]

Hair dye containing p-phenylenediamine and phenylmethyl pyrozolone is used to colour the hair and they are easily available. Hair dye was used for the first time in histopathology for inking the gross specimen.

On assessing the ease of application of different inking agents on the specimen, India ink needs repeated number of applications, because of its very thin consistency and in case of hair dye, it had to be mixed with water before application. So, acrylic colour was found easier to apply, the reason being its consistency. On comparing the drying time for different inking agents, acrylic has a longer drying time than hair dye and India ink.

Hair dye had an excellent visibility macroscopically on paraffin blocks when compared to that of India ink and acrylic. Yellow colour acrylic had least visibility on the paraffin block. On microscopic examination, India ink does not precisely show the margin [Figure 3]. Margins of the lesions were easily visible with acrylic colour –Vermilion [Figure 4]. While using yellow acrylic colour, they showed as black colour in microscopic sections [Figure 5]. So, not all the acrylic colours fulfil their role as inking agent when used for differentiating borders with different colours. On sections, hair dye did not colour the margins uniformly [Figure 6].

One of the qualities of the tissue marking dye is that, it should not contaminate the processing fluids. But all the inking agents which we used contaminated the processing fluids. Hair dye and India ink contaminated the dehydrating alcohol (50%) while acrylic colour contaminated xylene. This was in contradiction to the study done by Sachin C Sarode et al in 2015 where he found, acrylic had not contaminated the processing fluids.[3]

On microscopic examination, inking agent should not interfere with cellular details and should not pose problems in interpretation. In our study, India ink had penetrated the tissues and posed problem to some extent. This may be due to

the watery consistency of India ink when compared to acrylic colour and hair dye.

CONCLUSION

Within the limitations of this study, we conclude that commonly used hair dye and acrylic colours can be used as a substitute to India ink as marking dye for histopathology. Further Studies with larger sample size will validate these findings of the study. This will help the pathologists in handling of grossed specimen very effectively and identification of the surgical margins with ease.

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