Comparative evaluation of deparaffinization efficacy of kerosene, diluted lime water, refined sunflower oil and xylene

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ABSTRACT:

Aim: The primary objective of this study is to compare and correlate the deparaffinization efficiency of natural alternatives like kerosene, Diluted lime water, and refined sunflower oil and compare with routinely employed xylene for conventional hematoxylin and eosin staining (H&E) procedure by comparing their staining characteristics with xylene.

Methodology: Twenty-five paraffin-embedded tissue blocks were retrieved from the department archives. Four sections of 4 um thickness were made from each block and were randomly deparaffinized using four different agents (Xylene, Kerosene, Diluted lime water, and refined sunflower oil) Slides were examined for their cellular architecture and quality of staining for deparaffinization. Each section was then evaluated and scored blindly by two oral pathologists based on the criteria. The results obtained were analyzed statistically using SPSS Software and statistically compared using Mean, Standard deviation, and Chi-square tests.

Results and Conclusion: The statistical computation for our study demonstrated that there was a significant difference statistically among the four groups, with p values of 0.004 for nuclear staining, 0.001 for cytoplasmic staining, and 0.000 for clarity, uniformity, and crispness of the staining, respectively. The study's findings demonstrate the deparaffinizing effectiveness of kerosene and refined sunflower oil as well as a diagnostic trait similar to xylene. In addition to

being technically effective, features like being safe, affordable, requiring less time, and simple to handle and dispose of cannot be overlooked.

KEYWORDS: Deparaffinization, Xylene alternatives, Diluted lime water, Refined sunflower oil, Kerosene

INTRODUCTION:

In histopathology laboratories, numerous investigative analyses are performed on patient specimens. Poisonous chemicals, radio chemicals, and pathogenic bacteria are among the potentially hazardous compounds used in laboratories during such research methods. [1] Formalin and xylene are two commonly used chemicals in routine histopathology laboratory set up that are hazardous to human health and the environment. The health risks posed by xylene necessitate additional management efforts on the part of environmental health organizations and agencies.(2)

Xylene is a frequently employed solvent in the printing, rubber, and leather industries as well as a paint and varnish thinner. Xylene is a solvent for gutta-percha that is utilised in endodontic retreatment procedures as well as in histology laboratories for intermediate tissue processing steps such cleaning, deparaffinization, and cover sliding in dentistry. The tissue is more transparent due to the high solvency factor, which also allows for maximum alcohol displacement and enhances paraffin penetration. [3] However, xylene costs a fortune and harms people's health, causing hepatitis, chemical pneumonitis, depression, and anemia. Xylene exposure and handling are utmost during the dewaxing of sections. Exposure to xylene can occur through inhalation or ingestion, contact with the eyes, skin, or breathing it in. [4] Following this, it is predominantly broken down in liver by oxidizing a methyl group and conjugating it with glycine to create methyl hippuric acid, which is then eliminated in the urine. Xylene has been deemed toxic by the OSHA regulation standard, which urges its replacement with an equivalent, eco-friendly and profitable alternative to xylene. [5]

The quest for biofriendly xylene alternatives is of foremost importance due to its potential for hazard. This study was aimed at preserving the quality of tissue processing and staining while adopting more affordable, environmentally friendly options for xylene in tissue processing and staining procedures.

MATERIALS AND METHODOLOGY:

The study was conducted in the Department of Oral and maxillofacial pathology, Chettinad Dental College and research institute, and due ethical clearance was obtained from the ethical committee.

Twenty-five Formalin fixed paraffin embedded oral healthy tissue were retrieved from the archive blocks. The study was divided into 4 groups, each with a different deparaffinizing agent.

Group A—tissue sections stained with routine H&E stain using xylene as a deparaffinizing agent

Group B—tissue sections stained with xylene-free H&E stain using diluted lime water as deparaffinizing agent

Group C—tissue sections stained with xylene-free H&E stain using refined sunflower oil as a deparaffinizing agent

Group D—tissue sections stained with xylene-free H&E stain using kerosene as a deparaffinizing agent

Four sections of 4 μm thickness were made from each block. One section was stained with the conventional H&E method, where xylene was used as a deparaffinizing agent. The other three sections were stained by xylene-free H&E staining using 95% diluted lemon water, refined sunflower oil and kerosene as deparaffinizing agents, respectively. [Fig 1]

The slides were evaluated and scored blindly by two observers. Based on the grouping, parameters like nuclear staining, cytoplasmic staining (adequate = score1, inadequate = score0), uniformity, clarity, and crispness (present = score1, absent = score0) were all assessed on the slides. [Table 1] A total score of less than or equal to two was inadequate for diagnosis, while 3-5 was adequate.

RESULTS:

All the slides were examined microscopically with the fore mentioned criteria by individually two examiners and the values were tabulated and analyzed using Chi- square test. The following were the results analyzed and graphed based on the scoring given by two examiners.

An example of nuclear and cytoplasmic staining for all groups is shown in Fig 2. The scores obtained from the different groups were tabulated and statistically analysed using chi-square tests. The results of all groups are shown in Table 2.

Adequate nuclear staining was noted in 88% of group a, 76% of group b, 88% of group c, and 100 % of group d sections (p value =0.004). Adequate cytoplasmic staining was noted in 96% each in group a and group c, 76% of group b as compared with 100% in group b (p= 0.001).

Uniformity of staining was absent in 88% of group a and 96 % of group b and present in 72 % of group c and 68 % of group d (p= 0.000). Clarity of staining was present in 48% of group a and 20% of group b and in 64% of group c and 68% of group d (p =0.000). Crispness of staining was seen in 44 % of group a and 8% of group b, 32 % of group c and 84 % of group d (p = 0.000).

DISCUSSION:

Pathologists frequently use xylene during histoprocessing, deparaffinization, and mountant dilution. The tissue transparent nature and rapid removal of alcohols from tissues by xylene is makes them as an ideal dewaxing and clearing agent. [6] Deparaffinization is the removal of paraffin wax from slides prior to staining. Because wax has a melting point of around 70°C, an ideal deparaffinizing agent should be kept at that temperature for adequate deparaffinization. The quality of the stained sections, proper cellular architecture, and procedure time all played a role

in selecting an appropriate deparaffinizing agent. This irreplaceable nature of Xylene makes them as a promising deparaffinizing agent. [7]

As a result of new regulations, recent years and current research have been known for its inventiom of numerous alternatives to the xylene . Many studies are currently being conducted using a variety of biohazardous, cost-effective alternatives that improve the healthy laboratory environment without compromising staining quality or being adequate for diagnosis. The xylene-free method for paraffin sections was developed in 1995 at Vrinnevi Hospital in Falkeholm, with the hypothesis that xylene-free histological sections are comparable to conventional sections. [8] As a result, the current study attempted to replace xylene as a deparaffinizing agent with diluted lime water, refined sunflower oil, and kerosene.

An array of xylene alternatives has been generated in recent years as a result of new regulations. Many investigations are currently being conducted employing a variety of biofriendly, affordable alternatives that also improve the overall safety of the laboratory environment while maintaining appropriate staining quality for diagnosis. The xylene-free procedure for paraffin sections emerged at the vrinnevi hospital began in 1995 (Falkeholm) with the premise that xylene-free histological sections are analogous to conventional sections.[9]Hence, the present study aimed to replace xylene with diluted lime water, refined sunflower oil, and kerosene as deparaffinizing agents.

In our study, staining properties like nuclear and cytoplasmic staining, clarity of staining obtained using diluted lime water was inferior when compared with other alternatives and the crispness and clarity acquired was almost nil because of which the diagnostic adequacy obtained is also lessened. This is contradictory with the studies done by Prema et al [10] and Sravya et al. [1] This could be possibly due to the dilution of lime water or in the time period taken for deparaffinization. Lemon juice has a deparaffinizing agent from its organic solvent property that can be utilized to dissolve old wax. Lemon juice contains 5 to 6% acetic acid and has a pH of 2.2. The primary objective is that by raising the temperature from 90 to 94°C, wax will be removed from the slides and prevented from sticking again, aiding in the process of deparaffinizing the sections.

The slides stained using refined sunflower oil at the temperature of 60°c for 10 minutes yielded the comparatively superior results of cytoplasmic and nuclear staining, clarity, crispness and uniformity of the staining which attributes to the increased diagnostic adequacy. To our best knowledge, the study conducted using refined sunflower oil was limited. The results obtained were consistent with the study done by Sneha shish gosh et al. [11] The refractive index of RSO is (1.474) which is closer to that of tissue proteins (ranging between 1.33 and 1.4), which permit easy infiltration into the intercellular spaces of the tissues. Reduction in scattering of light and enhancement of the optical clearance of the tissues, transparency of the tissue is attributed to the refractive index.

Kerosene in our study showed superior staining characters when compared to that of other alternative agent that are employed in our study and they possessed maximum of diagnostically adequate slides. This was in consistent with the previous studies conducted by Janardhanam Dineshshankar et al. [12] Kerosene plays a crucial role in histopathology laboratories as a tissue-clearing agent, lengthening the tissue-clearing time relative to xylene in tissue processing, enhancing the accuracy of sectioning and staining, and demonstrating superior nuclear and cytoplasmic morphology, clarity, uniformity, and crispness of staining when compared to xylene.[13]

CONCLUSION:

Based on the findings of our study, we infer that 95% diluted lime water, refined sunflower oil and Kerosene can be employed as an alternative to xylene in deparaffinization. Apart from being technically efficient properties like eco-friendly, non-hazardous, economical, reduced time consumption, ease of handling and disposal cannot be neglected. Further studies with large sample sizes are to be done to validate these findings.

Images and tables

Fig 1 – Groups (Different Deparafinizing agents)

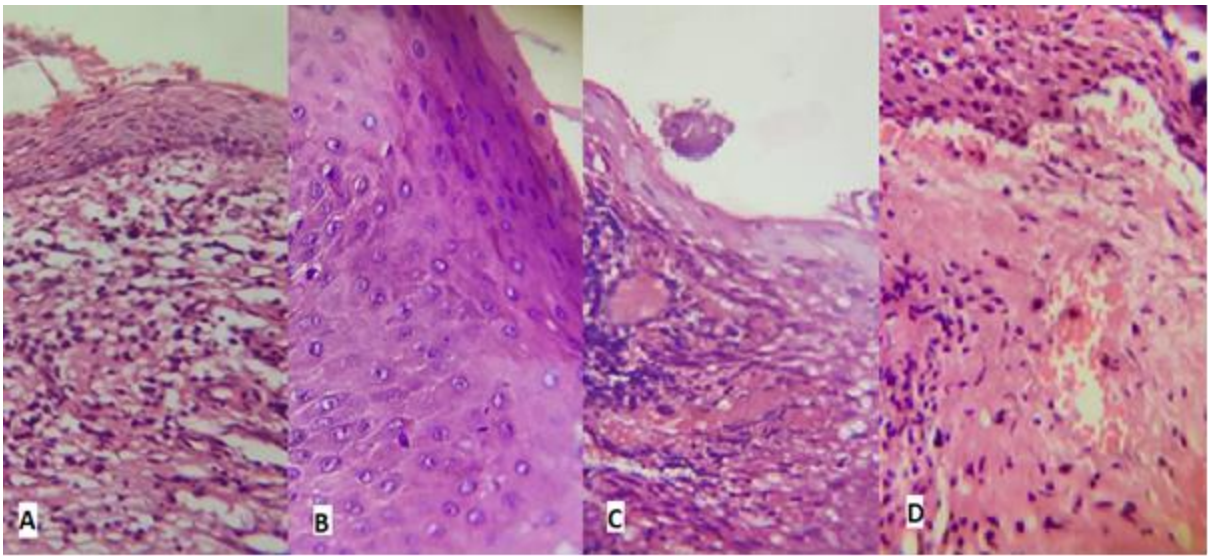

Fig 2 - H&E stained tissue (A – Group A Deparafinized using Xylene, B – Group B Deparafinized using 95% Dilute lime water, C – Group C Deparafinized using Refined Sunflower oil, D – Group D Deparafinized using Kerosene)

PARAMETERS	ADEQUATE	INADEQUATE
Nuclear staining	Score 1	Score 0
Cytoplasmic staining	Score 1	Score 0
PARAMETER	**PRESENT**	**ABSENT**
Uniformity of staining	Score 0	Score 1
Clarity of staining	Score 0	Score 1
Crispness of staining	Score 0	Score 1

Table 1 – Evaluation criteria and scoring

PARAMETER	GROUP I	GROUP II	GROUP III	GROUP IV	p VALUE
Nuclear staining					**0.004**
Adequate	22	19	22	25	
Inadequate	3	6	3	0	
Cytoplasmic staining					**0.001**
Adequate	24	19	22	25	
Inadequate	1	6	3	0	

Uniformity of staining					**0.000**
Present	22	4	12	23	
Absent	3	21	13	2	
Clarity of staining					**0.000**
Present	12	5	16	17	
Absent	13	20	9	8	
Crispness					**0.000**
Present	11	2	8	21	
Absent	14	23	17	4	

Table 2 - Evaluation of different deparafinizing agents

Legends

Table 1- Evaluation criteria and scoring

Table 2 – Evaluation of different deparaffinizing agents

Figure 1 – Groups (Different Deparaffinizing agents)

Figure 2 - H&E-stained tissue (A – Group A Deparaffinized using Xylene, B – Group B Deparaffinized using 95% Dilute lime water, C – Group C Deparaffinized using Refined Sunflower oil, D – Group D Deparaffinized using Kerosene)

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