Efficacy of Instant Transit Fixatives for Biopsied Soft Tissue Specimens: A Comparative Study

Minha Majeed Kak[1], Arya Jyoti[2], Priyanka Rastogi[3], Ritesh Kumar[4]

ABSTRACT

Introduction: Immediate and adequate tissue fixation is fundamental to good quality histological sections. Owing to the hazardous carcinogenic effects of 10% buffered formalin, its availability in clinics is questionable. As the search for formalin substitute has been enduring since it was proven as carcinogen, the present study was conducted with a novel approach to fixation, together with the scope of finding fixative properties of more commonly used reagents available at the clinics.

Materials and methods: commercially available fresh goat tongues were sliced and placed immediately in 10 different solutions as in Spirit, Ethanol (99.9%), normal Saline (0.9%), Betadine solution, H_2O_2 (6.5%), sodium hypochlorite (5%), Local anesthetic (2% lignocaine with adrenaline), Tap water and Distilled water for 12 hrs with 10% Buffered formalin as positive control. After the set time interval, the tissue specimens were further fixed in formalin for 24 hrs followed by routine tissue processing and Staining. The histological interpretations were a blinded procedure evaluated by two histopathologists. Results obtained were statistically analyzed and interpreted.

Results: Local Anesthetic proved to be an ideal instant transit fixative showing high quality tissue preservation with adequate staining results.

Conclusion: on the basis of results obtained, Local anesthetic solution can be used as an emergency instant transit fixative.

Keywords: 10% Neutral Buffered Formalin, Transit Fixatives, Local Anesthetic Solution, Normal Saline, Water.

INTRODUCTION

Tissue fixation, followed by its removal from the body, is one of the most essential determinants for the quality of histological sections primarily to prevent putrefaction and autolysis, and these changes once caused cannot be reversed.[1] Doctors and clinicians working in Government hospitals , clinics and remote areas see large number of patients in Outpatient centers and clinics and encounter suspicious lesions measuring equal to or more than 2-4 cm need to be incised for diagnosis to rule out Malignant Pathology.[2] Because of its good results, practicality and relatively low cost, 10% buffered formalin (4% buffered formaldehyde), is the most widely employed universal laboratory fixative for tissues[3] but it is not routinely kept in the clinics owing to its undesirable effects that include its carcinogenic and irritating potential.[4] Due to unavailability of Formalin, in certain situations, the valuable tissue specimen get wasted and discarded due to lack of knowledge regarding importance of biopsy[1]. For such situations, we intend to find out an alternate instant transit solutions (carrying/transport media) that are easily available in all the clinical setups and can be used for preserving tissues, before they are transferred to formalin at the nearby histopathology lab. Hence, the present study was conducted to evaluate the efficacy of tissue fixation with other different regularly found agents in the dental clinic, i.e., Local anesthetic solution (LA), Normal Saline (NS), Tap Water (TW), Distilled water (DW), Betadine (B), Hydrogen peroxide (HP),Spirit, Ethanol (E) and Sodium hypochlorite with that of 10% neutral-buffered formalin till transferred to the laboratory.

MATERIAL AND METHODS

This study was conducted in the department of oral and maxillofacial pathology Kothiwal Dental College and Research centre Moradabad. Fresh tissue specimens (goat tongue) were obtained from the slaughter house and no animal was harmed for the purpose of the study. Each tongue tissue was collected and it's anterior and posterior- third was discarded. The middle portion of the tongue was retained and used for the study purpose. The tissue were grossed into 5 equal parts and were immersed directly in Spirit, Ethanol (99.9%), normal Saline (0.9%), Betadine solution, H_2O_2 (6.5%), sodium hypochlorite (5%), Local anesthetic (2% lignocaine with adrenaline), Tap water and Distilled water, and two bits were placed immediately in formalin container that served as control at 0 hour and in the mentioned solutions for 12 hrs.

The tissues were subsequently transferred to 10% normal buffered formalin solution for overnight fixation at room temperature. After fixation, tissues were grossed from the centre and then tissue processing steps were carried out followed by sectioning and staining. 4 µm tissue sections were obtained and stained with routine Hematoxylin and Eosin, using standard protocols[5].

The prepared slides were then subjected to scoring by three independent observers (oral pathologists) individually

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for the histological assessment of fixation under light microscope (at X4, X10 and X40) (Fig 1-8). The pathologists scoring the slides were blinded to the fixation protocol. The Histomorphological criteria examined were:

1. Nuclear details
2. Cytoplasmic details
3. Cellular outlines
4. Intercellular bridge visibility
5. Loss of cohesiveness
6. Overall morphology
7. Retraction artifacts
8. Vacuolization artifacts
9. Wrinkling
10. Tissue folding
11. Missing tissue fragments
12. Staining quality

Each histomorphological criteria was rated on a scale of 0-5.

0. Very poor

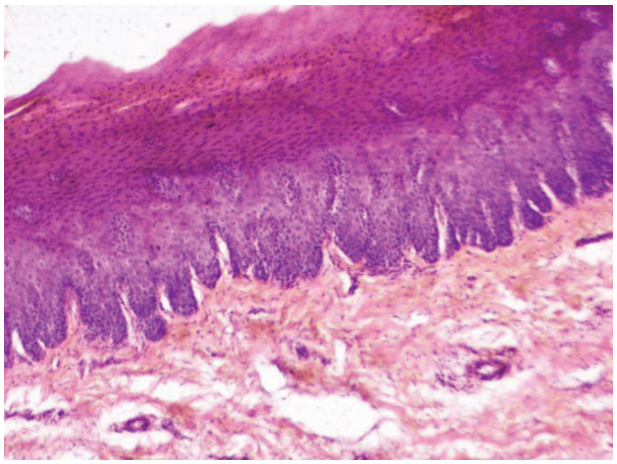

Figure-1: Betadine

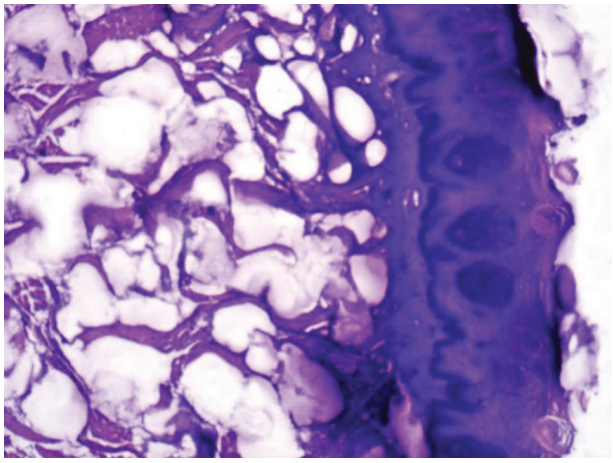

Figure-4: Hydrogen Peroxide

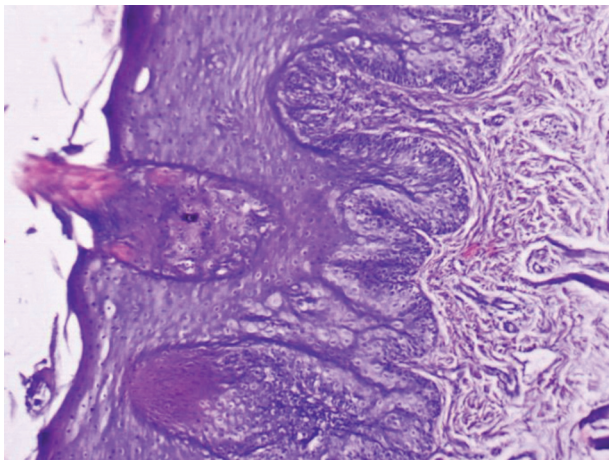

Figure-2: Distilled water

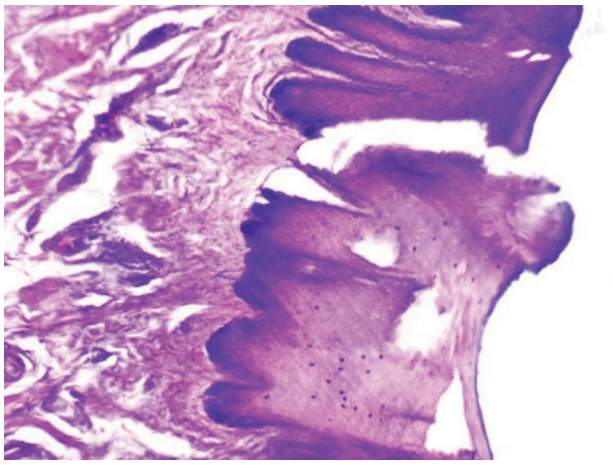

Figure-5: Sodium Hypochlorite

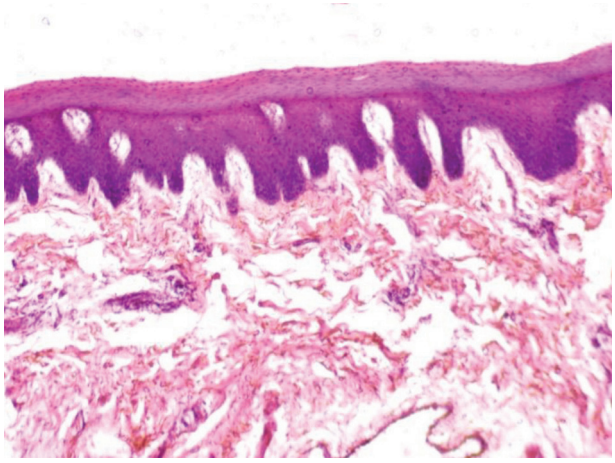

Figure-3: Ethanol

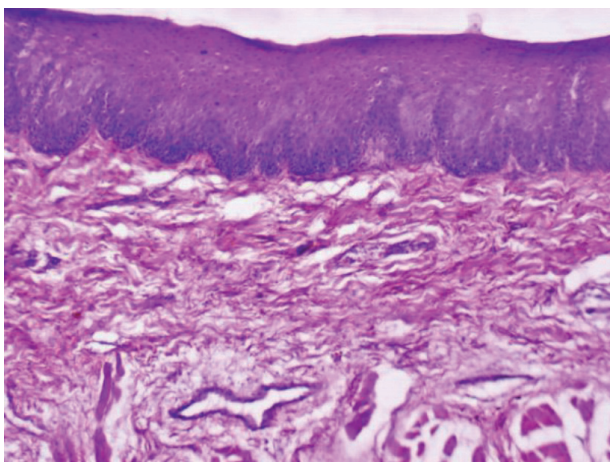

Figure-6: Local Anesthetic

1. Poor
2. Average
3. Satisfactory
4. Good
5. Excellent

The average values of the three examiners were recorded and tabulated (Table 1).

Depending upon the scores obtained, total scores were taken and the values were used to group the tissues into following:

Scoring Criteria

Excellent	61 – 72
Good	49 - 60
Satisfactory	37 – 48
Average	25 – 36
Poor	13 – 24
Very Poor	0 – 12

The data hence achieved (Table 1) was statistically analyzed and discussed in light of prevailing scientific information, and appropriate conclusions were drawn.

RESULTS

From the data obtained it was observed that the fixation of the tissues in 10% buffered formalin gave ideal results which acted as a positive control for the whole study. All the transit fixatives were able to preserve the tissue till a time gap of 12 hrs before they were transferred to formalin. Tissues placed in local anesthetic, normal saline and distilled water showed excellent overall results while tissues placed in sodium hypochlorite, Betadine and Hydrogen peroxide showed overall poor results with difficulty in sectioning rendering the tissues hard to section. Summing up the overall results, the tissue preserving ability was in the following order: Formalin > Local Anesthetic > Normal Saline > Distilled water > Tap water > Ethanol > Spirit > Hydrogen Peroxide > Betadine > Sodium Hypochlorite.

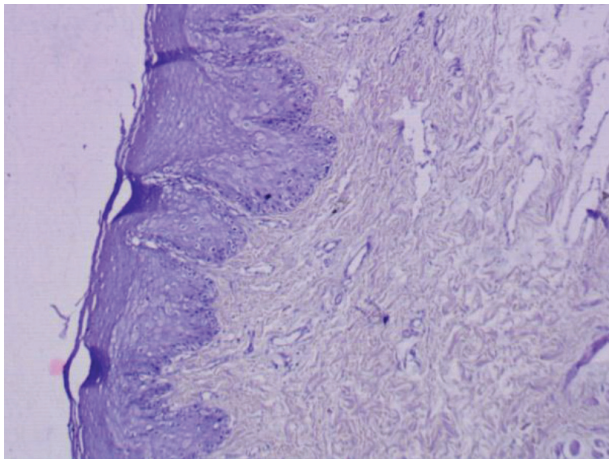

Figure-7: Normal Saline

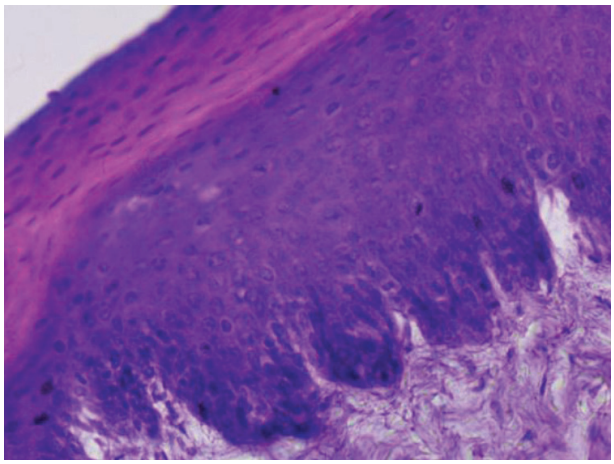

Figure-8: Spirit

Formalin	Local anesthetic	Normal saline	Distilled water	Tap water	Ethanol	Spirit	Hydrogen peroxide	Betadine	Sodium hypochlorite	Transit fixative	
5	5	5	3	2	2	1	0	3	0	Nuclear details	Histomorphological criteria
5	5	4	4	3	3	1	2	2	0	Cytoplasmic details	
5	4	3	3	2	2	1	1	1	1	Cellular outlines	
5	5	4	2	3	1	1	1	1	0	Intercellular bridge visibility	
5	3	3	3	3	2	2	2	2	2	Loss of cohisiveness	
5	3	3	3	3	2	3	1	1	2	Retraction Artifacts	
4	3	2	3	1	1	2	1	1	1	Vacuolization	
4	4	3	1	2	2	2	1	1	1	Wrinkling	
5	4	2	2	1	2	3	0	0	1	Tissue folding	
5	4	4	3	1	3	1	1	1	0	Missing tissue fragments	
5	5	4	4	3	2	1	3	0	0	Staining quality	
5	5	3	4	3	3	2	2	2	2	Overall morphology	
58	50	40	38	27	25	20	18	15	10	Score (72)	

Table-1: Showing Histomorphological Criteria of Various Fixative Agents

DISCUSSION

Fixation is a critical step in the preparation of histological sections. Errors in fixation can cause irreversible damage to the tissue specimen. No matter how much care is subsequently taken in tissue processing, Microtomy and staining, the morphological and histochemical information obtainable from the specimen will be compromised. It is therefore important that the principles and practice of tissue fixation are applied to obtain quality slides. For practical purposes fixation aims to prevent or arrest the degenerative processes which commence as soon as a tissue is deprived of its blood supply.

It is important to realize that a fixative will initially produce a number of changes to the tissues that is usually an aqueous environment. These will include shrinkage, swelling and hardening of various components. Thus, the total effect of a particular fixative on a tissue should be assessed after it has been processed, sectioned and stained.

Carrying Medias are considered as holding agents rather than fixatives because they do not chemically alter tissues. They are often used to transiently prevent desiccation of tissues. They are used as a buffer system in cell culture media and aid in maintaining the optimum physiological pH (roughly 7.0-7.4) and osmotic pressure providing the cells with water and inorganic ions.

In our study transit medias were chosen on the basis of their easy availability in dental clinics and hospitals set ups.

In surgical pathology, 10% neutral buffered formalin has been the gold standard since ages.[1,2] Due to its carcinogenic effects, the safety hazards of formalin remain a major subject of concern for its routine use in the laboratories.[1]

Several studies have been conducted to search for a substitute to formalin. Normal saline and Distilled water were considered unsuitable as transit media by Khoo in 1995 and Oliver et al in 2004 as they result in poor fixation and artifactual changes.[6,7] In contrary, Siraj A et al advocated use of NS And DW as fixatives in their study[8] Severe tissue alterations making diagnostic evaluation difficult was seen with fixation with water.[9]

Poor fixation results with betadine, spirit and water were also obtained by a study conducted by Rajnikanth M et al[2]

LA is considered to interact with tissue membranes yet its action as fixative at molecular level is not clear.[1] Fixation results with Local Anesthetic were found to be excellent by studies conducted by Kasetty M et al [1] and Rajnikanth M et al.[2]

CONCLUSION

The clinicians or the dentists must be familiarized to different transit fixatives or transporting agents which could be used in dental clinics or hospitals till the proper fixative is made available. When using a carrying media, clinician should specify the type of carrying media and the time it is kept in it, so that when it is received by the pathologist the time lapse can be calculated and thus anticipated alteration in tissue architecture would be kept in mind by the pathologist at the time of diagnostic interpretation.

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