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ORIGINAL RESEARCH

Microwaves: A Revolution in Histoprocessing

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ABSTRACT

Background and Aim: Pathologists are under constant pressure for instant and reliable diagnosis. The manual procedures employed in private laboratories and institutional setup for histoprocessing and staining are laborious and intense. Thus, this study aims to evaluate and compare the microwave tissue processing and staining with the conventional methods which are in vogue.

Materials and methods: Of the formalin fixed tissue biopsies received by our department, 30 specimens were randomly picked and subjected to grossing. Each specimen was cut into equal halves, each half was processed and stained by conventional method while the other by the microwave method. The entire procedure was blinded and evaluated by four observers based on the criteria of Mahesh Babu et al (2011): Cellular clarity, cytoplasmic details, nuclear detail and color intensity. The results were statistically analyzed using Chi square test and kappa.

Results: The overall time employed for microwave processing was 2 hours and for conventional methods it was 7 hours, while H and E staining by microwave process took 16 minutes and 45 seconds and it took 31 minutes and 20 seconds by the conventional process. The diagnostic ability of microwave method yielded promising results and was less time consuming.

Conclusion: Microwave processing and staining yielded quicker and better results compared to the routine methods. Therefore, Microwave can serve as a quicker and a reliable diagnostic method for a pathologist.

Keywords: Microwave, Conventional, Processing, Staining.

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INTRODUCTION

Conventional tissue processing is traditionally been a gold standard procedure practiced since ages involving dehydration, clearing and infiltration.[1] This permits tissue samples to be embedded in a solid medium for sectioning, following which staining is done to confer contrast and make tissue components visible facilitating the diagnosis.[2,3] These procedures are tedious and time consuming. Rapid processing and staining of histological specimen is of paramount importance for early diagnosis and management on a 1 day basis.[4]

Diffusion is the key factor in histoprocessing permitting chemicals to permeate into a tissue faster thereby reducing the time. Microwave oven generates heat from within (internal heating) and warms the object uniformly and hence hastens tissue processing.[5] The microwave was earlier restricted only to domestic usage and with time they have been introduced in the field of histotechnology.[2] The usage of microwave guards against the hazardous effects of xylene thereby permitting a conducive work environment. Hence, the practice of microwave assisted tissue processing, has brought about a revolution and has become a boon to pathologists.[4]

Though, there are many studies done on microwave assisted tissue processing, there are only a handful of studies based on microwave staining, hence this study was undertaken to evaluate and compare microwave tissue processing and staining with the conventional method.

MATERIALS AND METHODS

Microwave oven (Samsung Model no: CE104VD, Input -1250W, Output-900W), Microwave glass jar – 200 ml 5 jars, Ethyl alcohol, Isopropyl alcohol, Xylene, Paraffin wax, Harris hematoxylin, 0.5% HCl, Eosin were the materials used.

Sample selection: Thirty tissue samples were randomly selected from those received by the department of oral pathology MS Ramaiah Dental College and Hospital, Bangaluru. Scalpel biopsies (1 × 1 cm upto 3 cm in size, 5 to 8 mm thickness) fixed in 10% buffered formalin were included in the study. Grossly lacerated, hard tissues and surgical procedures other than scalpel biopsies were excluded in the study.

Each specimen was cut into equal halves, one half was processed and stained by conventional method while the other half was processed and stained by the microwave method. The procedure for conventional processing and staining (Tables 1 and 2) was according to our department protocol while microwave processing, and staining (*see* Tables 1 and 2) was as per Mahesh babu et al (2011).[4] The microwave temperature was standardized at 100 W.

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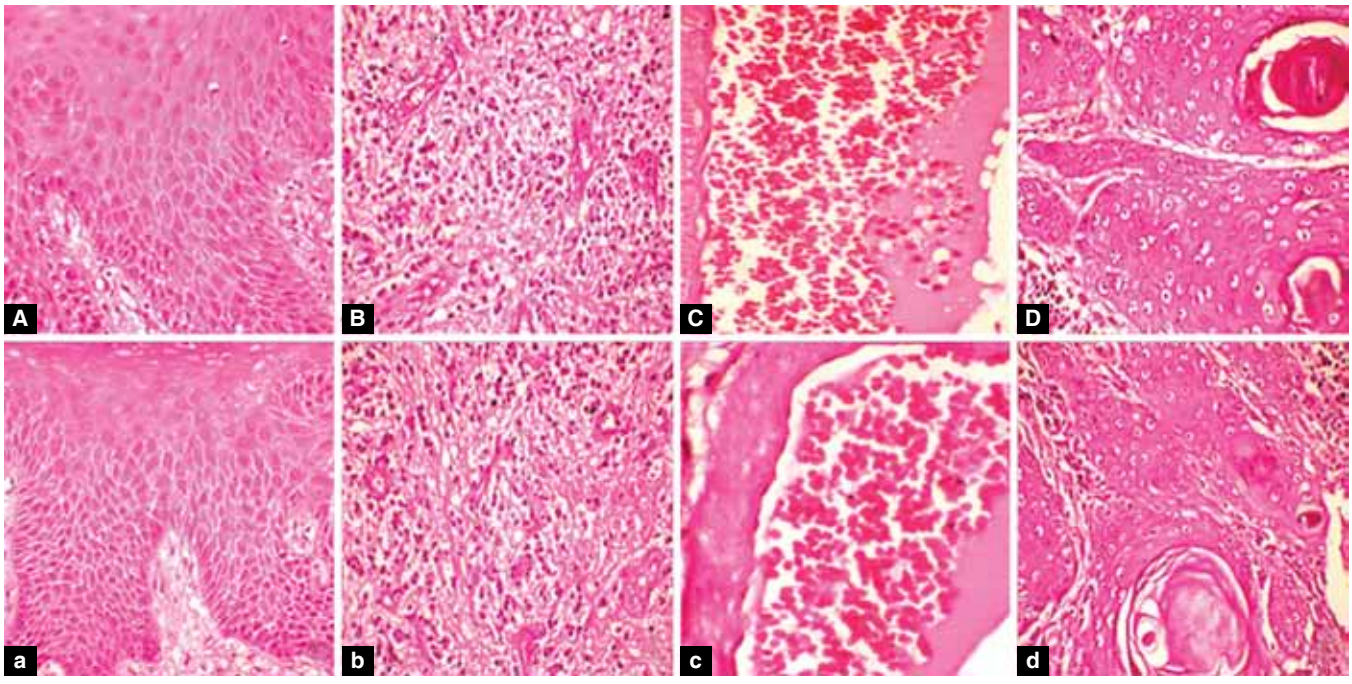
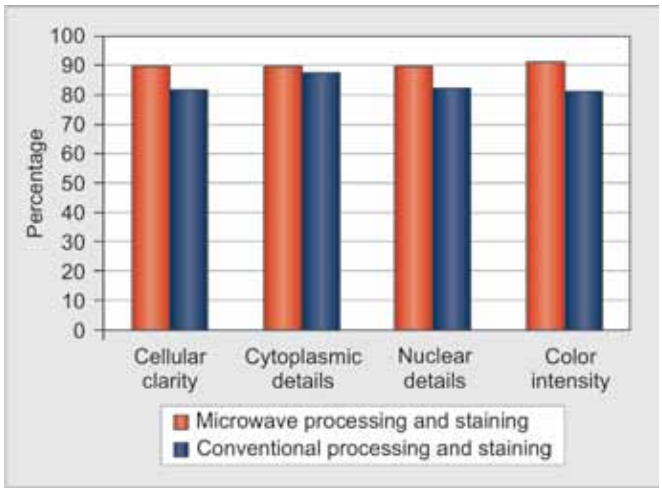
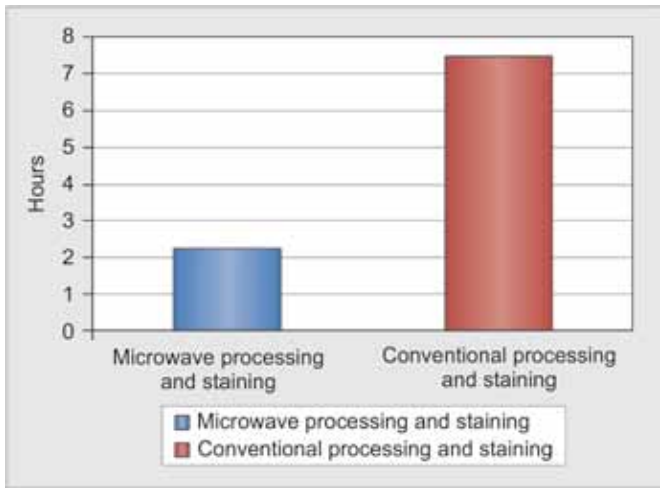


Figs 1A to D: Microwave (capital letters) versus conventional method (small letters); A, a: Cellular clarity, nuclear details, cytoplasmic details and color intensity noted in the epithelium; B, b: Inflammatory cells; C, c: Red blood cells; D, d: Squamous cell carcinoma



Graph 1: Representation of correlation of data of microwave processing and staining with that of the conventional



Graph 2: Representation of the time taken for microwave and conventional processing and staining

Table 1: Conventional and microwave processing technique

Steps involved	*Conventional processing*	*Time required*	*Microwave processing*	*Time required*
Dehydration	70% ethyl alcohol	1 hour	100% isopropyl alcohol	30 minutes
	80% ethyl alcohol	1 hour		
	90% ethyl alcohol	1 hour		
	100% ethyl alcohol	1 hour		
Clearing	Xylene	30 minutes	100% isopropyl alcohol	30 minutes
	Xylene	30 minutes		
Impregnation	Paraffin wax	1 hour	Paraffin wax	30 minutes
	Paraffin wax	1 hour	Paraffin wax	30 minutes
Total time		7 hours		2 hours

The entire procedure was blinded and evaluated by four observers using criteria by Mahesh babu et al[4] (Table 3) and analyzed using chi square test and kappa statistics.

RESULTS

The cellular clarity, nuclear, cytoplasmic details and color intensity (Graph 1, Figs 1A, a) was better in microwave

Table 2: Conventional and microwave staining technique

Steps involved	*Conventional staining*	*Time required*	*Microwave staining*	*Time required*
Hydration	70% ethyl alcohol	5 minutes	100% isopropyl alcohol	5 minutes
	60% ethyl alcohol	5 minutes		
	Tap water	5 minutes	Water wash	2 minutes
Staining	Harris hematoxylin	10 minutes	Harris hematoxylin	2 minutes
Blueing	Running water	5 seconds	Running water	2 minutes
Differentiation	0.5% HCl	5 seconds	Acid alcohol	5 seconds
Water wash	Tap water	5 minutes	Water wash	5 minutes
Stain	Eosin	1 minutes	Eosin	30 seconds
Water wash	Tap water	5 seconds	Tap water	5 seconds
Dehydration	100% ethyl alcohol	5 seconds	100% isopropyl alcohol	5 seconds
Total time		31 minutes 20 seconds		16 minutes 45 seconds

Table 3: Criteria for evaluation of quality of slides

Parameters	*Evaluation*	*Scores*
Cellular clarity	Excellent	4
Cytoplasmic details	Good	3
Nuclear details	Average	2
Color intensity	Poor	1

processed and stained tissues than the conventional protocol. The p value was 0.068, which shows that there is no statistical difference between the two methods and kappa statistics was 0.900, which showed high agreement between the observers.

The time taken (Graph 2) for microwave processing and staining was 2 hours 16 minutes and 45 seconds, while it took 7 hours 31 minutes and 20 seconds for the conventional method.

DISCUSSION

The recent trends dominating in the field of histopathology are immunohistochemistry and molecular assays while histoprocessing and staining have taken a back seat.[4] Keeping in mind, rapid diagnosis, these issues have been overcome by the introduction of microwaves in pathology.

A potential application of microwave energy in histotechnology was first recognized by Mayers in 1970, who successfully fixed tissue with a microwave generator used in physiotherapy.[2] This later broadened the horizon, with applications in tissue fixation, histoprocessing, staining, techniques for electron microscopy, antigen retrieval, frozen and immune-techniques.[6]

Microwave heating depends on oscillating or exciting polar or charged molecules. Microwaves force dipolar molecules of proteins to rotate through 180° at the rate of 2.45 billion cycles per second. The molecular kinetics induced, results in generation of instantaneous heat that is proportional to the energy flux and continues until radiation ceases. The microwaves which stimulate polar molecules causes collision with the adjacent molecules causing a part of the rotational energy to be transferred through them, as heat. This effect occurs simultaneously throughout the whole material being microwaved and thus hastening the procedure.[5]

The merits of microwave have surpassed the age old conventional methods with respect to a shorter processing time, lesser degree of denaturation of nucleic acids and exclusion of noxious chemicals like xylene.[7,8] Also, domestic microwaves are readily available, affordable and have been experimented for tissue processing with appreciable results.[5] Hence, we opted the use of the same.

A power mode of 100 to 300 W was opted for histoprocessing and staining in the study to combat the tissue damage although it comes with a variable range (100-850 W). This is in conjunction with the studies of Raju et al[5] and Mahesh Babu.[4] Also microwave glass wares were preferred as metallic utensils ignite sparks, favoring Mahesh Babu's study.[4]

Although maximum tissue load is permissible up to 25 samples/load we employed 6 tissues/load and a uniform thickness of 5 to 8 mm throughout with diffusion of fluids being the priority.[9] This was in accordance with Prasad et al's[1] study which reflects that diffusion is dependent on the thickness and not on the length and breadth of the specimen.

The conventional tissue processing protocol is quite cumbersome with respect to histoprocessing while microwave tissue processing is much simplified and employs isopropyl alcohol alone with dual effect of dehydrant and clearing as the residual alcohol gets evaporated by the microwave energy during impregnation thereby eliminating the need for a separate clearing procedure. This in turn cuts the cost and materials with hazardous effects of xylene at bay. This is in accordance with the study of Raju[5] and Pritam[7] et al.

The scores of the evaluated slides were collated and the diagnostic ability was assessed based on the observations of: cellular clarity, cytoplasmic and nuclear details.

Microwave method was noted to be superior to the routine method (*see* Graph 1, Figs 1 A, a). This was in consonance with the studies of Kok and Boon,[10] Mahesh[4] and Pritam[7] et al

and in contrast with the studies of Morales[11] et al, Chaudhari[12] et al and Mathai[6] et al as both the procedures yielded same results.

Microwave method excelled when compared to conventional with regards to the color intensity (*see* Graph 1, Figs 1A, a). This was in consonance with the findings of Mahesh babu,[4] Hopewood[14] and Leong[13] et al study. Also, Hopewood[14] and Leong[13] et al noted eosinophilia in tissues stained by microwave which could be reversed by altering the time of staining with eosin.

In the present study, we could differentiate the types of inflammatory cells such as plasma cells, lymphocytes, etc. by the microwave method (Figs 1B, b). which is similar to that of Hopewood[14] et al's study. Also, the integrity of red blood cells was well maintained with the use of microwave (Figs 1C, c) which is similar to Prasad[1] et al study and in contrast with the studies of Hopwood[14] and Leong[13] et al resulting in lyses of red cells.

Incidentally the dysplastic features encountered in one of the tissues remained same in both the methods with similar results of Hopewood,[14] concluding that the pathological diagnosis, including malignancy, can be given satisfactorily with microwave processed slides.

Most significantly, microwave processing and staining was less time consuming (7 hours 31 minutes and 20 seconds) when compared to the conventional protocol (2 hours 16 minutes and 45 seconds) (*see* Graph 2). This would permit diagnosis on the same day and thus facilitate management on a 1-day basis.[8] This was in agreement with studies conducted by Ralph[8] et al, Morales[11] et al and Mahesh[4] et al.

CONCLUSION

Microwave processing and staining was noted to be many folds superior to the conventional. Hence, we recommend the adoption of microwave technique in histoprocessing on a routine basis. In addition the burden on the laboratory technicians is reduced to a great extent.

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