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RESEARCH ARTICLE

DOMESTIC MICROWAVE BASED RAPID TISSUE PROCESSING VERSUS CONVENTIONAL TISSUE PROCESSING – A COMPARATIVE STUDY IN SMALL BIOPSIES

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ABSTRACT

Turnaround time has now-a-days become increasingly important. Conventional tissue processing is a time consuming procedure. Recently microwave tissue processing has been shown to reduce the processing time significantly. Laboratory microwaves are sophisticated costly instruments to be used routinely especially in developing nations like India. In the present study kitchen microwave was used for tissue processing to evaluate its usefulness in terms of quality and time saving in comparison with conventional one. 126 paired specimens were evaluated in a blinded fashion by two pathologists. Each H&E stained slide is evaluated based on the criterias laid down by Kumar *et al* 2014. Statistical evaluation of the two procedures using a chi-square test showed no significant difference in the sections produced by both the procedures.

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INTRODUCTION

Microwaves are discovered by Percy Spencer in 1945 (Kumar, 2014). These are electromagnetic waves having frequencies 300MHz to 300 GHz. Microwaves were commonly used to heat the cooked food within a fraction of time required by other methods of heating. The principle of working of microwave ovens being they produce microwaves that excite molecules in materials to rotate resulting in production of heat. The heat thus produced reduces the viscosity of the liquid thereby increasing the rate of diffusion of reagents in and out of the tissue. The heating produced in microwaving is uniformly throughout the whole material which is not in conventional heating (Hicks, 2005). This heating reduces the time required for the three basic steps of histoprocessing namely dehydration, clearing & impregnation by increasing the rate of diffusion. This improves the turnaround time of the procedure which is of major importance now-a-days in terms of patient care. Cost factor is an important issue in health care especially in developing countries like India where routine use of Laboratory microwave is a remote possibility in remote health centres. The present study is aimed at evaluating the usefulness of domestic microwaves for histoprocessing in terms of (i) turnaround time & (ii) quality of microscopy in comparison to conventional overnight histoprocessing.

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MATERIALS AND METHODS

It was a prospective study conducted at Balaji Diagnostics & Research Centre, Brahmapur during the period July 2013 to March 2015. Randomly selected 72 small biopsies (<2 mm thick & <20 mm long) (Table 1) constituted the study group. Each sample is divided in to two groups: Group A for conventional processing & Group B for microwave processing. All biopsies were received in 10% Neutral Buffered Formalin and are allowed to fix in the same solution for 6 hours. Before processing in the domestic microwave all the samples are rinsed in Normal Saline for 30 minutes to remove as much formalin as possible to prevent formalin fumes while microwaving. Group A samples were routinely processed using the overnight schedule (Table 2) and Group B specimens were processed using domestic microwave the schedule of which is given in Table 3. Processed tissues were embedded in Paraffin & 3 to 4 µm sections were obtained using rotary microtome and stained with H&E stain. The following observations were recorded.

- Turnaround time for tissue processing – time taken for the formalin fixed tissue to be ready for paraffin embedding.
- Quality – Each paired stained slide was evaluated in a blinded fashion by 2 Pathologists based on the criterias used by Kumar *et al.* 2014. (Table 4)

Table 1. Types of tissue taken for study (n = 72

1	Endometrium	32
2	GI endoscopic biopsy	35
3	Core needle biopsy	5
Total		72

Table 2. Overnight Processing Schedule for 'Group a' Samples

1	10% Buffered saline	3 – 4 hours
2	70% Isopropanolol	1 hour
3	90% Isopropanolol	1 hour
4	Absolute Isopropanolol	1 hour
5	Absolute Isopropanolol	1 hour
6	Absolute Isopropanolol	1 hour
7	Xylene	30 minutes
8	Xylene	30 minutes
9	Paraffin bath	1 hour
10	Paraffin bath	1 hour
Total duration		11 – 12 hours

Table 3. Microwave processing short schedule for 'group b' samples

FIXATION: Normal saline at 62^0C – 30 seconds		
1	100% Ethanol at 67^0C	5 minutes
2	100% Isopropranolol at 74^0C	5 minutes
3	Molten Paraffin (Preheated) at 80^0C	5 minutes
Total duration		15 minutes

Table 4. Criterias used to analyze the stained slides

		Distinct	Indistinct
Cellular morphology			
1	Integrity of tissue / stroma	Well preserved	Poorly preserved
2	Cellular outline	Crisp	Blurred
3	Cytoplasmic staining	Crisp	Blurred
4	Red cell lysis	Absent	Present
5	Secretory products	Appreciated	Not appreciated
6	Inflammatory cells	Appreciated	Not appreciated
Nuclear morphology			
7	Nuclear outline	Well defined	Smudged
8	Nuclear chromatin	Appreciated	Not appreciated
9	Nucleoli (if present)	Appreciated	Not appreciated
Inference: "1" – Overall cytoplasmic & nuclear morphology distinct & "0" - when it is indistinct.			

Table 5. Comparison of quality of microscopy

Method	Indistinct		Distinct	
	Cellular	Nuclear	Cellular	Nuclear
Microwave	8 (11.11)	6 (8.33)	64 (88.89)	66 (91.67)
Conventional	12 (16.67)	11 (15.28)	60 (83.33)	61 (84.72)
Total	20 (13.89)	17 (11.81)	124 (86.11)	127 (88.19)

All recorded results were statistically evaluated using the chi-square test.

Observations

Turnaround time

The average turnaround time for tissue processing for the control group was 11 to 12 hours while the same for study group (microwave processed) were 15 minutes only. This resulted in a quick same day reporting possible.

Fixation

Microwaving tissue bits completely immersed in NS at 62^0C for 30 seconds was shown to have better preservation of morphology especially in the epithelial tissues.

Tissue processing

Microwave assisted histoprocessing does not require the clearing step with Xylene in comparison to the routine schedule as the alcohol used for dehydration is removed by microwave heat before paraffin enters. So it eliminates the use of toxic chemicals like Xylene.

Quality for Microscopy

All the paired H&E stained histopathology slides were independently evaluated by two pathologists in a blinded fashion using the criteria given in the Table 4. The differences observed in both the procedures are statistically insignificant (p-value for cellular features and nuclear features were 0.335 & 0.197 respectively) indicates that microwave assisted processing produces histosections of quality comparable to that produced by the time consuming routine schedule.

Others

In addition to reducing the turnaround time the microwave was also useful in quick staining for special stains like PAS, Masson Trichrome stain whenever required.

DISCUSSION

With modernization most of the traditional medical techniques are replaced by newer ones, the most noticeable exception is histotechnique which is still largely unchanged requiring 1 to 2 days for the purpose. In recent years the use of microwave for the purpose has shown to reduce the time required to few hours. The prime aim of histotechnique is to prepare thin sections with preservation of life like morphology of tissue that are to be stained and observed under microscope for tissue diagnosis. To make it possible to take thin sections of tissue all the constituents of the tissue must be of equal density. Paraffin wax has been universally accepted as the embedding medium of choice for the purpose because of its suitable physical properties. Paraffin is liquid when heated and solid but soft at room temperature when it is easy to take thin sections. (Ralph Rohr *et al*., 2001) The morphology of the tissue is preserved in life like manner by fixing the tissue in chemicals known as fixatives, most accepted and hence routinely used chemical for the purpose is formalin. The basic principle of tissue processing is to impregnate the tissue with paraffin so that paraffin fills the tissue in subcellular levels and then the tissue is embedded in the same paraffin to prepare a solid block that is sufficiently firm enough to fix it in a microtome yet soft enough to take thin sections of 2 – 5 µm. This is achieved in three steps, (Kumar *et al*., 2014) dehydration – where water from subcellular level is removed so that water immiscible paraffin can enter and fill the space.

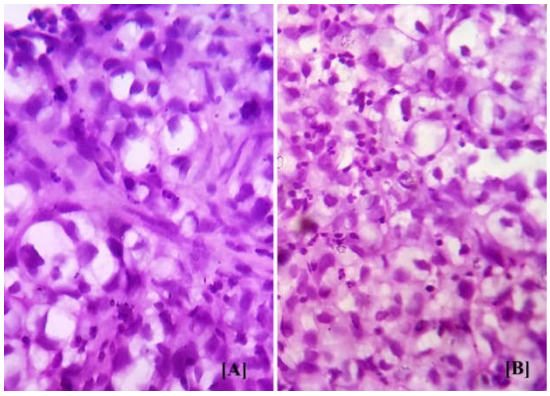

Figure 1.

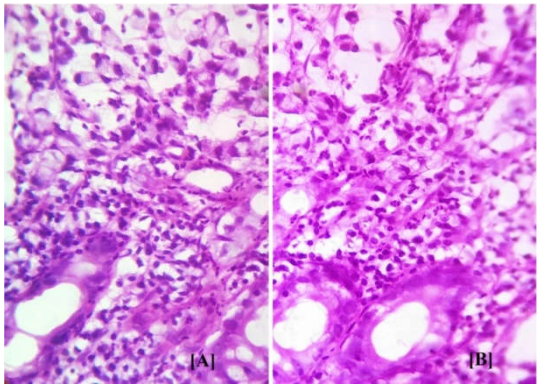

Figure 2.

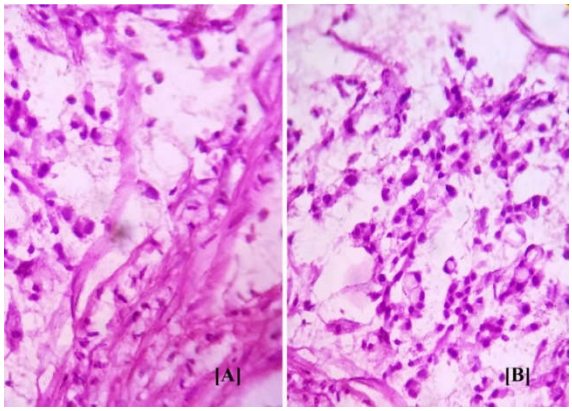

Figure 3.

This is done by using graded concentrations of alcohol, (Hicks, 2005) clearing – is the step that follows the dehydration where a solvent like xylene is used which removes the alcohol now present in the tissue and allows paraffin to enter followed by (Ralph Rohr *et al*., 2001) impregnation and embedding with paraffin wax. As the exchange of liquids takes place by simple diffusion the procedure is very time consuming. Factors like heat, vaccum and agitation has been shown to accelerate the process of diffusion and hence reduce the time required. Microwaves are electromagnetic non-ionizing waves in the frequency range of 300 MHz to 300 GHz (Willis and Minshew, 2002; http://www.ebsciences.com/papers/microwaves.htm#imulator; Sivadas *et al*., 1996; Kok and Boon, 1990). The mechanism of action of microwave is it rotates the bipolar molecules like water which in turn produces heat which accelerates the process of diffusion of the chemicals in and out of the cells. And all these occurs throughout the tissue in a uniform manner. Microwave-assisted tissue processing has been studied since 1970 (Ralph Rohr *et al*., 2001). But since 1986 the use of microwave ovens in various areas of tissue processing has been studied as evident from a number of publications in the field (Ralph Rohr *et al*., 2001). The advantages noted with microwave technique in various studies are (a) accomplishes rapid fixation of even large specimens like mastectomy, colon, lymph node without use of formalin thus preventing the laboratory workers exposure to noxious formalin fumes (Leony and Duncis, 1986). (b) satisfactory results with immunofluorescence in kidney biopsy specimens fixed in a saline medium by microwave irradiation (Mac-Moune *et al*., 1987). (c) better quality frozen sections when the tissue on a glass slide covered with few drops of a solution containing ethyl alcohol and polyethylene glycol is radiated with microwave prior to the procedure (Kok *et al*., 1987). (d) the procedure is environment friendly as it eliminates the use of toxic chemicals like xylene as the residual alcohol is "boiled out" by microwave energy used to heat liquefy paraffin. (Ralph Rohr *et al*., 2001) (e) microwave technology has been accepted in the field of immunohistochemistry starting from antigen retrieval to staining.

The other important advantage of microwave technique is significant reduction in turnaround time. This is most important in cases of malignancy. To cite an example, an endoscopic biopsy taken in the morning from a suspected case of carcinoma stomach can be reported around noon in the same day and a definitive surgery can be performed in the same day. Besides it has been documented that this microwave procedure overcomes the constraints like difficulties of interpretation of procedures that have a quick turnaround time such as frozen section. In accordance to other studies the present study has also observed no significant difference in quality of microscopy of tissue sections prepared by microwave technology as compared to routine method. This is evident from the observation that the p-values obtained for cellular morphology and nuclear morphology was 0.335 & 0.197 respectively are statistically insignificant. Though not included in the study protocol it has also been observed that the quickness and quality of special stains such as PAS, Masson's trichrome was better.

Conclusion

Microwave tissue processing is very useful in reducing the turnaround time in histopathology practice without compromising the quality of microscopy. Domestic microwave can be used for the purpose whenever financial constraints are there.

Legends to Figures 1, 2 & 3: Photomicrographs comparing the quality of sections obtained in both the procedures. (A) – conventionally processed & (B) – microwave processed.

Consent: A written informed consent has been obtained from the patients for publication of this work. The original copy of the same is available with the corresponding author which can be submitted to the editor-in-chief of this journal on demand.

List of abbreviations:

H&E: Hematoxylin & Eosin

Competing interests: The authors declare that they have no competing interests. There is no financial or non-financial competing interest with any party, person or institute.

Author's contribution: MKP carried out concepts & design, literature search, participated in the study, data acquisition, data analysis & manuscript preparation, will stand as guarantor also. AC carried out concepts & design, literature search, manuscript review. DB participated in the laboratory study, data acquisition & manuscript review.

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