ORIGINAL RESEARCH PAPER

Pathology

# MICROWAVE TECHNOLOGY IN HISTOPATHOLOGY AND ITS COMPARISON WITH THE CONVENTIONAL TECHNIQUE.

KEY WORDS:

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|---|---|
| **Dr Swagatika Senapati** | Assistant professor, Dept. of Pathology, S.C.B. Medical College and Hospital , Cuttack. |
| **Dr. Subhashis Mishra** | Assistant professor, Dept. of Pathology, S.C.B. Medical College and Hospital , Cuttack. |
| **Dr. Debi Prasad Mishra** | Professor, Dept. Of Pathology, MKCG Medical College And Hospital, Berhampur. |
| **Dr. Kailash Chandra Agarwal*** | Professor, Dept. Of Pathology, Vss. Medical College And Hospital, Burla. *Corresponding Author |

## INTRODUCTION

Keeping in pace with the changing scenario towards modernization in the field of medical technology, traditional techniques have been replaced by newer ones. But histotechniques in histopathology more or less still remains the same with just a few changes. For almost 100yrs, the steps followed to prepare tissues for microscopic evaluation have remained unchanged but the time consumed by these steps have reduced from several days to merely one or two days and now with the advent of microwave tissue processing it has come down to few hours.

We have come a long way from the time the conventional tissue processing, was proposed in the 19th century to frozen sections to automatic tissue processor to the successful application of microwaves in the field of histotechniques for fixation and then processing.

The practice of microwave assisted tissue processing brought about a reassessment of traditional concepts. The paradigm of numerous sequential steps of tissue fixation, dehydration, clearing and impregnation ingrained as a tradition of conventional methods has been greatly simplified in all reported microwave methods.

Microwaves, which were invented by Percy Spencer in 1945, created a small buzz and soon became an integral part of our daily lives and their application in pathology laboratory are an inevitable outcome. Thus, a novel histoprocessing method for paraffin section was developed and fast processing was possible due to stimulated diffusion of the heated reagents.

The microwave used for histotechniques works on the principle that electromagnetic field causes excitation of molecules which brings about its rotation. This produces energy in the form of heat from within the materials. This heat enhances the rate of diffusion of fluids in and out of the tissues blocks or sections even more effectively in contrast to conventional heating.

This study uses microwaves for the processing of biopsy tissues and analyse the cellular and nuclear morphology as well as staining characteristics. Comparison is done with the conventional technique using the same parameters.

The method reported herein reproducibly yields histologic material of similar and satisfactory quality to that provided by time-honored conventional processing. It has many advantages including expediency, safety, potential for preservation of molecular integrity of specimens that might be used in subsequent studies, and improvement in the workflow of laboratory, permitting the preparation of diagnostic material during the day time.

The routinely used microwave oven in a histopathology lab is a laboratory microwave oven. This ,apart from all the advantages it has, like maximum output of 2000-3000 watts, an inbuilt source of adjustable temperature probe, facility for ventilation etc is extremely expensive as compared to a domestic microwave oven.

The ready availability of domestic microwave ovens and their ease of operation makes this form of non ionizing radiation an attractive method. The purpose of this study was to assess the reliability of the domestic microwave oven as against the established routine processing and to establish the domestic microwave as a valuable tool for rapid reporting without compromising on the quality.

## METHODOLOGY

Whirlpool Microwave oven Model no.MS-1921HE Input- 230V AC, 50 Hz; Output- 700W, Microwave frequency 2450 MHz. Power consumption 1000Watts outside dimensions 480mmx275mmx336 mm.

### a) Sample selection

Specimens for this study were selected randomly from those received in the department of Pathology V.S.S. Medical College and Hospital, Burla over a span of two years . Only soft tissue was preferred for this study

1. The sample size was 1cmx3mm-1.5cmx3mm
2. The thickness of 1mm-3mm was taken for microwave processing
3. The tissue was then divided into equal halves; one was processed in microwave and the other was processed conventionally.
4. 101 surgical biopsy and autopsy tissues were used in the present study.

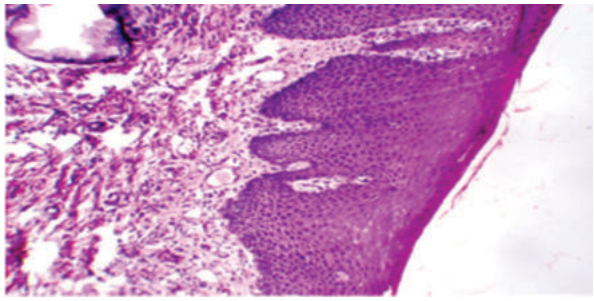


**Figure 1b – Microwave - Chronic cervicitis, tissue architecture maintained**

**b) Fixation**

10% formalin was used as a fixative

**c) Conventional tissue processing**

The processing was started at 9 am; the cassette was kept in water for 1 hour to remove formalin. All processing was done at room temperature, except for impregnation and embedding .

| STEPS | REAGENT/ PROCESSING FLUID | TIME |
|---|---|---|
| FIXATION | 10%Formalin | 6hr |
| DEHYDRATION | 70% isopropyl alcohol | 2hr |
| | 80% isopropyl alcohol | 2hr |
| | 95%isopropyl alcohol | 2hr |
| | 95%isopropyl alcohol | 2hr |
| | 100%isopropyl alcohol | 2hr |
| | 100%isopropyl alcohol | 2hr |
| CLEARING | Xylene | 1hr |
| | Xylene | 1hr |
| IMPREGNATION | Molten paraffin | 2hr |
| | Molten paraffin | 2hr |
| | TOTAL TIME-24hrs | |

**d) Microwave tissue processing**

1. Standardization of the procedure

A pilot study was done in which tissues of varying sizes and texture were processed following the same schedule (protocol-1).Smaller tissues faired better while the larger ones needed longer processing time hence larger tissues were processed at lower power and for a longer time (protocol-2)

**Table 3-Protocol1**

**Pre-fixed tissue for more than 12-24 hours** **Total no.36**

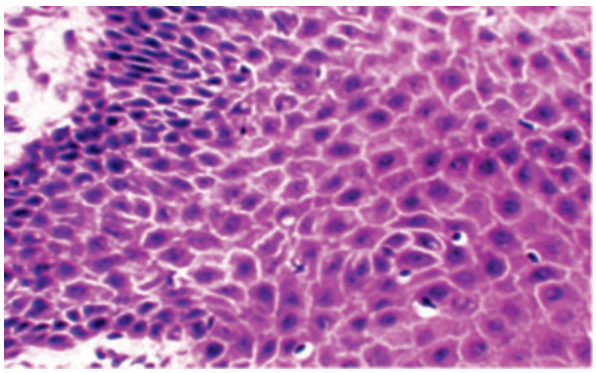
| Processing steps | Reagent/processing fluid | Microwave power | Time |
|---|---|---|---|
| Dehydration | 70%ethyl alcohol | 450 watts | 4 mins |
| Clearing | 100%isopropyl alcohol | 450 watts | 4 mins |
| Impregnation | Molten paraffin wax | 750 watts | 7 mins |
| Total time-15 mins | | | |

**Table 4-(Protocol-2)** **Total no.65**

| Processing steps | Reagent/processing fluid | Miicrowave power | Time |
|---|---|---|---|
| Dehydration | 99%isopropylalcohol | 100 watts | 30 mins |
| Dehydration | 99%isopropylalcohol | 100 watts | 30 mins |
| Impregnation | Molten paraffin wax | 100 watts | 30 mins |
| Impregnation | Molten paraffin wax | 100 watts | 30 mins |
| Total time-2hours | | | |

**2) Embedding and Section cutting**

Both groups conventionally and microwave processed tissues following impregnation in paraffin wax, were embedded in paraffin wax using L blocks . Blocks of both group of tissues were placed in the refrigerator for 10mins to ensure solidification and easy sectioning. Conventional - Squamous epithelium, cellular / nuclear morphology distinct . (H&Ex40)



**3) Staining with H&E and Reticulin.**

In the present study 101 pairs of H&E stained sections were prepared 10 tissues processed using protocol 2 were used

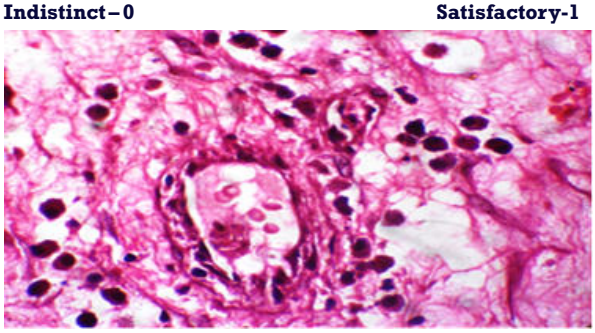
**4) Studying of sections**

| These paired H&E slides were then evaluated by general pathologist for following 5 | Mitosis if applicable | Seen | Not Seen |
|---|---|---|---|
| Sl.no. | Staining | | |
| 1 | Poor | Not stained/unevenly stained, has artifacts | |
| 2 | Satisfactory | Details not visualized,but suitable for diagnosis | |
| 3 | Good | Good contrast,visibility of details,brilliant staining | |

**GRADE**

**Distinct - 1** **Poor - 0**

**Indistinct – 0** **Satisfactory-1**



Conventional- Inflammatory nasal polyp, good erythrocyte integrity and well preserved inflammatory

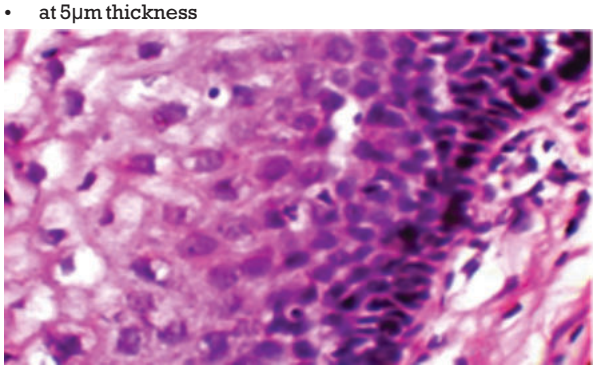
**Besides the above parameters,**

**Tissue architecture -**was also studied looking for

- Fragmentation of tissue sections
- Integrity of epithelium and connective tissue

**Difficulty in sectioning of tissues**

- at 5µm thickness



Conventional - Squamous epithelium, cellular / nuclear morphology distinct . (H&Ex400)

After evaluation by all four observers the results were subjected to statistical analysis .

**DISCUSSION**

The new technique of processing tissue using a microwave employed in this study represents a major change from conventional tissue processing. The ease of application and speed of this technique has significantly reduced turnaround time in diagnostic labs for the past 3 decades. Initially application of microwave techniques into histotechnology was not accepted but nowadays is growing in its popularity and versatility.

The prime proposal in our study was to undertake microwave processing as per schedule mentioned in protocol I which was also adopted by Suri V et al[30] But that did not work well with tissues of larger size and of varying consistency and so we planned for protocol II which produced excellent results.In our study we followed protocol I for small biopsy

and smaller size tissuesand for larger tissues protocolII

In microwave technique isopropanol is an effective intermedium as the problem of slow diffusibility can be overcome by microwave heating. Isopropanol is less toxic and cheaper as well[7a,Ban]. We used only isopropanol in our study which was both a dehydrating and clearing agent. Xylene has a high boiling point and low microwavability and so during clearing with xylene the residual ethanol boils and causes tissue damage and during impregnation also xylene could not be boiled out and hence retarded the diffusion[33b]

In this study, the microwave processed tissue sections had good cellular morphology which included nuclear cytoplasmic contrast, stroma, secretory products ,cellular outline, and were distinct in most of both conventional and microwave processed tissue. This indicated that microwave processing did not affect the cellular morphology in any way. This was in consonance with study done by Mathai AK et al [39]

Both the microwave and routine processing tissues showed some amount of shrinkage. The overall shrinkage of microwave processed tissue was more .The shrinkage noted in both routine and microwave processed tissues is due to the shrinkage effects of alcohol where there is replacement of water molecules from the hydrophilic sites of the peptides chains in the denatured proteins. The shrinkage noted was negligible and did not interfere with the diagnosis; the slight increase in shrinkage could be due to the heat used in the study.[10]

Boon et al found that in microwave processed tissues the epithelium was of better quality, while the stroma had a slightly different appearance, in that it appeared to be slightly more condensed focally. Similar results were seen in this study where the epithelium showed excellent nuclear and cytoplasm contrast and the intercellular bridges were also appreciable. Focal condensation of connective tissue is of no importance in diagnostic pathology, as explained by Kok[8]

There was no significant difference between nuclear size and shapes. The staining characteristics were discernable, as seen in cases studied by Mathai et al .[

Merits of microwave techniques

Microwave irradiation has several advantages over routine methods from the perspective of the laboratory personnel providing them with safe and clean environment. It eliminates the need for xylene in tissue processing and may reduce or eliminate the need for formalin when stabilization is done with 1M saline From the perspective of the final product, microwave irradiation substantially shortens the time from specimen reception to diagnosis. In our experience, this reduced preparation time (2-3 hours, including fixation, processing, microtomy, and staining) allows same-day tissue processing and diagnosis of small biopsy specimens without compromising the overall quality of the histologic section.

Right from pre-cancerous lesions to malignancy, reactive lesions to benign tumors, microwave technique has shown a remarkable result without losing the architecture and morphology of the cells.

Microwaves, because of their wide range of applications (electron microscopy, antigen preservation) can be used in diagnostic laboratories as a means of cost containment. The profitability of any diagnostic laboratory would be increased by the use of this technique as a large batch of samples can be handled in a single day and it will also be a boon for the technical personnel whose work practices and lifestyles would change for the better and this is something which defies statistical analysis.

The potential applications and versatility of microwave processing methods are unattainable with conventional procedures. The method reported herein reproducibly yields histologic material of similar quality to that provided by time-honored conventional processing. It has many advantages, including expediency, safety, potential for preservation of molecular integrity of specimens that might be used in subsequent studies, and improvement in the workflow of the laboratory, permitting the preparation of diagnostic material during the day at family friendly hours.

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