Original Article

Comparative Study of Tissue Processing and Staining Using Microwave and Routine Method

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Abstract

Background: Conventional tissue processing in histopathology requires 16-24 hours, creating longer turn-around time in routine histopathology laboratory work. Microwave-assisted tissue processing (MTP) has emerged as a promising alternative for rapid histopathological diagnosis.
Objective: To evaluate the diagnostic utility and efficiency of domestic microwave-assisted tissue processing and staining in comparison to routine histopathology method.
Methods: This prospective, cross-sectional analytical study compared conventional and microwave-assisted tissue processing using 350 paired tissue specimens (700 total) over three years. Tissues were sectioned into two equal halves, with one processed conventionally and the other using a Samsung domestic microwave (300W for processing, 180W for staining). Quality parameters including cellular details, cytoplasmic preservation, nuclear clarity, and staining characteristics were blindly evaluated using a scoring system (0-40 points).
Result: Microwave processing reduced turnaround time from 16 hours 20 minutes to 81 minutes (processing: 67 minutes vs 16 hours; staining: 14 minutes vs 20 minutes). Statistical analysis revealed superior performance in cellular outline, nuclear-cytoplasmic contrast, nuclear/nucleolar clarity, chromatin preservation, and color intensity ($p<0.05$). Both methods showed equivalent performance in tissue integrity and nuclear membrane clarity. Special stains (PAS, Alcian blue, Masson's trichrome, Fontana-Masson) and immunohistochemistry demonstrated comparable results.
Conclusion: Domestic microwave-assisted tissue processing produces diagnostic quality equivalent or superior to conventional methods with reduction of turnaround time. This technology enables same-day diagnosis, particularly beneficial for critically ill patients and small biopsies, representing a paradigm shift toward efficient, environmentally conscious histopathology practice.

Keywords: microwave; tissue processing; tissue staining; histopathology; turnaround time

Introduction

Histopathological diagnosis of specimens is greatly dependent on good sample preparation and staining. Both of these processes are governed by diffusion of fluids and dyes in and out of tissue, which is the key in tissue processing and staining. Shorter turn-around time is important in this age of managed care, commitments and health care services.[1] Thus, rapid processing of histopathologic tissue is becoming increasingly desirable to fulfill the needs of clinicians treating acutely ill patients. For more than 100 years there has been one main method of processing tissues to obtain thin sections for microscopic examination is referred to as conventional tissue processing.[2] Other new technology such as microwave

assisted tissue processing and staining was assessed in present study. Domestic microwave was used for modifications of routine processing and staining techniques of biopsy specimen by generation of heat. Microwave method of tissue processing is an economical procedure which reduces use of noxious chemicals as well as turn-around time.[3] The purpose of present study is to document the usefulness of a microwave assisted accelerated method for tissue processing and staining protocol developed indigenously in the laboratory and to compare it with microscopic examination of tissue sections by focusing on cellular, cytoplasmic, nuclear details and staining characteristics.

Materials and Methods

Present study is cross-sectional analytical prospective study. The study protocol had received institutional ethics committee approval (CEC Reg No: ECR/85/Inst/GJ/2013/RR-16). Written inform consent was taken. The study evaluated two tissue processing and staining techniques—conventional and microwave-assisted methods—using 350 paired tissue specimens (700 total) collected over three years. Tissue sections size ranging from 0.8 x 0.8 x 0.6 cm to 1.5 x 1.5 x 0.8 cm were taken from specimen and fixed for at least 24 hours in 10% neutral buffered formalin. Small biopsies (<1x1x0.6 cm) and bony hard tissues were excluded from this study.

Each tissue block was sectioned into two equal halves (3–4 mm thickness). One half underwent routine processing and staining, while the other used microwave-assisted methods. Samsung microwave oven model MS23K3513, with a maximum output of 800 W was utilized for present study (Figure 1). Tissue processing was performed at 300 W (38% power) over six consecutive 10-minute cycles in a adequately ventilated room. Reagents were changed for reuse after each cycle to prevent overheating, and temperature was monitored by laboratory mercury thermometer at the end of cycle 1 and cycle 4. Temperature was maintained between 70–85°C. For Hematoxylin and Eosin (H&E) staining, the microwave was operated at 180 W (23% power).

Figure 1: Samsung microwave oven model MS23K3513.

In the microwave method, tissues were dehydrated in 300ml methanol (two 10-minute cycles at 300 W), followed by 300ml isopropyl alcohol (two 10-minute cycles at 300 W) in borosil jar. Paraffin infiltration was performed using molten paraffin wax in two 10-minute cycles at 300 W. Clearing agents were not required due to the evaporation effect of microwave heating. Tissue sections were cut from paraffin blocks, mounted on egg albumin-coated slides, and stained. Turnaround time for processing and staining by microwave method was 67 minutes and 14 minutes respectively.

For conventional processing, tissues were dehydrated in Isopropyl alcohol, cleared in xylene, and paraffin-embedded. H&E stain done by hematoxylin for three minutes, acid alcohol differentiation, and eosin for 30 seconds, followed by dehydration and mounting. Staining protocol of H&E required total 20 minutes. Turnaround time for processing and staining by conventional method was 16 hour 20 minutes respectively.

Slides were prepared by both the staining methods. For coding, both the slides were labelled as A or B randomly with specimen number. In same manner, further samples had been labelled progressively as number progresses i.e Specimen 25 was labelled as 25A and 25B where evaluator did not know by which method the tissues has been processed and slides were stained. They were blindly evaluated by two experienced pathologist using a scoring system. Four parameters—cellular details, cytoplasmic details, nuclear details, and staining characteristics were evaluated which also includes ten sub-parameters (Table 1) each scored out of four for a total of 40. Grading was as follows: Poor (<10), Average (11-20), Good (21-30), and Excellent (31-40).

Statistical analysis included descriptive statistics (mean, standard deviation) and independent t-tests to compare the two methods. A 95% confidence interval ($p<0.05$) was applied to assess significant differences in staining quality and efficiency between conventional and microwave-assisted techniques.

Table 1: Scoring system for evaluation of staining characteristics.

Criteria	Poor - 1 mark	Average - 2 marks	Good - 3 marks	Excellent - 4 marks
Cellular details				
Cellular outline				
Clarity				
Integrity of tissue				
Cytoplasmic details				
Nuclear cytoplasmic contrast				
Eosinophilia/ Granularity				
Nuclear details				
Clarity of nucleus & nucleoli				
Clarity of nuclear membrane				
Clarity of chromatin				
Staining characteristics				
Colour intensity				
Uniformity				

Results

In Gross examination, maximum tissues were of grey color and firm in consistency. volume of each section was assessed before and after processing for length, breadth and width. Percentage of shrinkage was calculated. The average volume of tissue was 1.34x1.15x0.38= 0.585 cu mm that is 100% volume of tissue. After tissue processing by routine method, the average volume of tissue was 1.23x1.04x0.29=0.37 cu mm. Thus, volume after processing was 63.31% by routine method which showed shrinkage of 100%-63.3%=36.7%.

The tissue processed by microwave method showed mean volume of 1.21x1.03x0.27=0.34 cu mm. Thus, volume after processing was 57.5% by microwave method which showed shrinkage of 100%-57.5%=42.5%. Shrinkage of tissue by routine method was 36.7% and by microwave method was 42.5%. Thus, difference of shrinkage between two methods was 42.5%-36.7%=5.8% which showed shrinkage of tissue was slightly more in microwave method than routine tissue processing method.

All tissues were assessed as Grade IV (Excellent)-Marks between 31 to 40. In histopathological examination. Cellular outline, nuclear cytoplasmic contrast, clarity of nucleus and nucleoli, clarity of chromatin and colour intensity were better in microwave method as compared to routine method and the difference was statistically significant. Clarity of cellular details, integrity of tissue and clarity of nuclear membrane were equally preserved in both the methods. There was slight difference in which Eosinophilia/granularity and uniformity in staining were better by routine method as compared to microwave method, however the difference was not statistically significant (Figure 2).

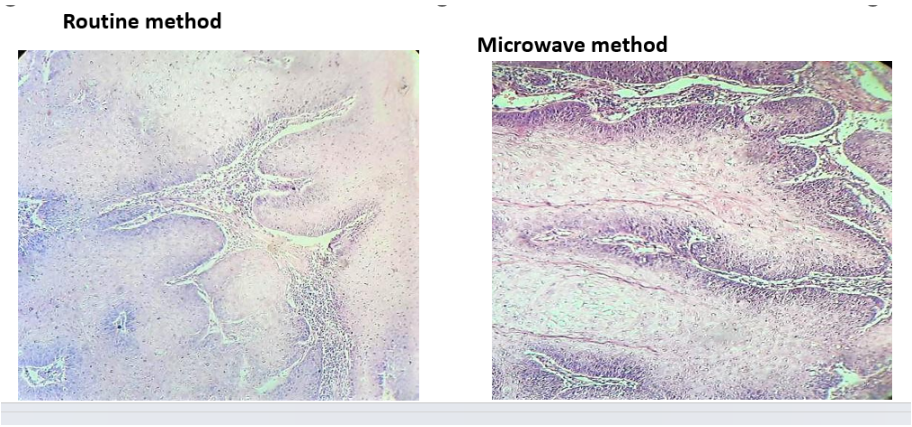

Figure 2: Comparison of H & E stained section of squamous cell carcinoma. (Low power magnification-10X)

Various special stains like Periodic acid Schiff, Alcian blue, Massion trichrome and Fontana Massion were performed on tissues processed by both the methods and the results were comparable. Thus, tissue components retain their specific chemical characteristics not only by routine processing but also by microwave processing (Figure 4). The immunohistochemistry was performed on tissues processed by both the methods and the results were comparable (Figure 5).

Thus, overall working time for Routine method was 16 hour and 20 minutes while for microwave method was 81 minutes.

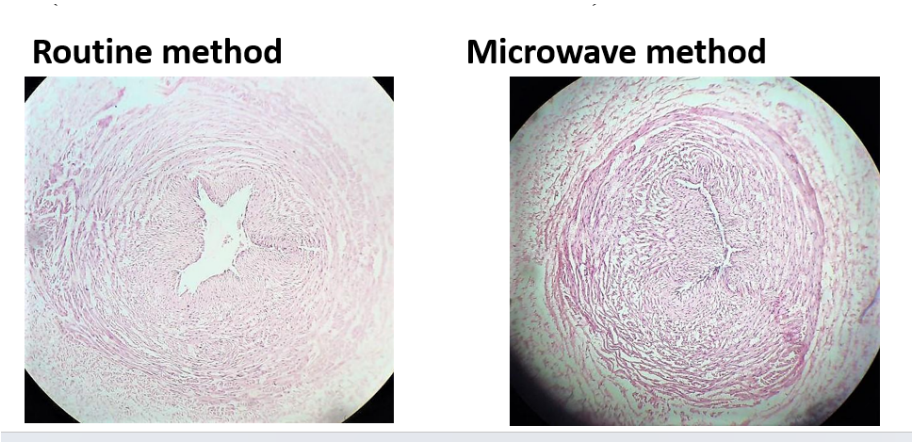

Figure 3: Comparison of H & E stained section of umbilical cord. (Low power magnification-10X)

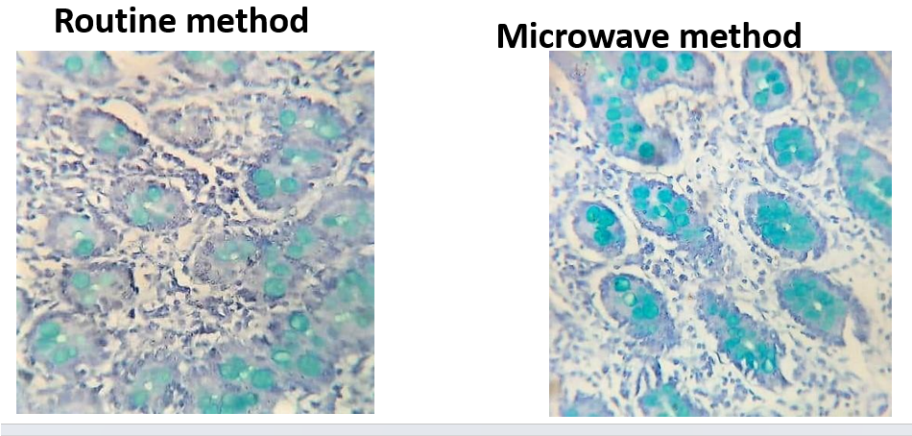

Figure 4: Comparison of alcian blue stained mucin of intestinal glands. (High power magnification-40X)

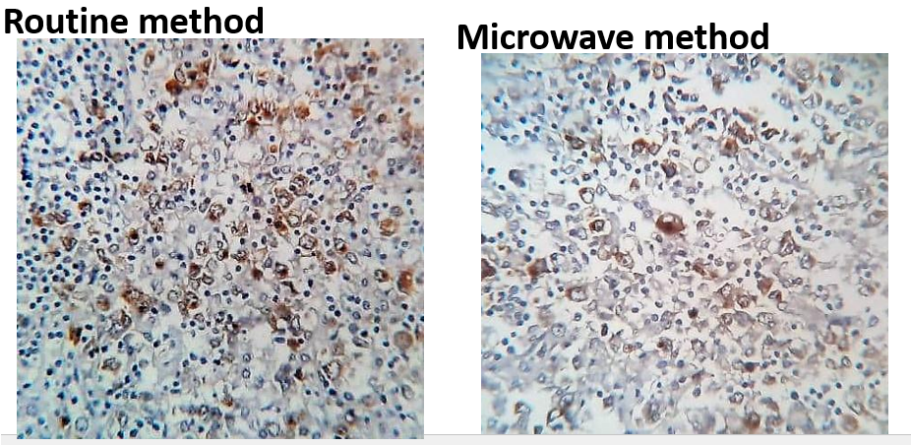

Figure 5: Comparison of immunohistochemistry of Hodgkin lymphoma (CD 30 Biogenex clone HRS4).

Thus, domestic microwave method has a significantly shorter processing and staining time.

Discussion

Rapid processing of histopathologic material is becoming increasingly desirable to fulfill the needs of clinicians treating acutely ill patients. Traditional methods for quickly processing tissues take 4 to 5 hours, which can delay treatment for some critically ill patients. Microwave processing shortens this time, allowing rapid histopathologic tissue processing and staining.[4, 5] Thus, patients with critical illness or neoplastic diseases can be diagnosed early and therapy can be initiated.

FinNie O et al. demonstrated that a 62-minute microwave protocol could yield results comparable to the 12-hour Conventional Tissue Processing cycle. The heat generated from microwave allows reagents like alcohol and paraffin to penetrate the tissue

matrix at a significantly accelerated rate which leads to significant reduction in Turn-around time.[1]

Unlike conventional methods that rely on external heat conduction microwave energy provides volumetric heating that stabilizes reagents internally. This mechanism prevents the formation of a peripheral "crust" or the shrunken interface often observed with traditional external heating sources. 5.8% more tissue shrinkage by microwave processing was even as microwave start heating the tissue from within. Hence, there was no alteration in morphology for microscopic evaluation and it did not compromise marginal evaluation in malignancies. Mild marginal shrinkage without microscopic alteration in tissue was also reported in study done by Saurabh et al.[6]

In a study by Rao and colleagues, samples from the buccal mucosa and gingiva were used to evaluate microwave-assisted tissue fixation, processing, and staining. The goal was to see if this method could replace the standard formalin-fixed paraffin-embedded technique for tissues of different thicknesses. Using microwave tissue fixation in routine processing and staining, the slides were ready in 113 minutes. This enabled same-day processing and diagnosis of small biopsy samples without affecting the quality of the histological sections.[7]

In study done by Munkunda et al., microwave-stained slides showed no loss of cellular and nuclear features, had uniform staining, and were of high quality. The overall quality of the microwave-stained tissue sections was found to be better than that of the routinely stained sections in most cases. The microwave oven used in the study had a maximum power output of 800 Watts, and the authors used the lowest setting of 100 Watts throughout the study. The processing time was 2 hours. Although some studies suggest that microwaves can be operated at higher power levels, ranging from 100 to 200 Watts, which can reduce processing time from 1 to 2 hours to as little as 5 minutes, shrinkage was observed in both routine and microwave-stained tissues. The amount of shrinkage was minimal and did not affect the diagnostic process. The slight increase in shrinkage seen in microwave-stained tissues may be due to the heat used during the procedure.[8]

Conventional processing requires Xylene as a clearing agent because paraffin is not miscible with alcohol. Tupsakhare S et al has done significantly advanced work for elimination of Xylene by using chemistry of Microwave energy.[9] Xylene is a known neurotoxin and carcinogen, and its disposal is an environmental burden. As observed in the studies of Tupsakhare S and Chandy et al.[9, 10], the use of Isopropanol at elevated microwave temperatures allows the direct transition to paraffin. The microwave energy causes the alcohol to evaporate rapidly as the paraffin infiltrates the tissue, effectively "skipping" the need for a clearing agent. This not only makes the laboratory a safer, "green" environment but also reduces the cost per slide by eliminating one of the most expensive reagents in the lab.

Priya et al.[11] compared the efficacy of domestic microwave oven in staining oral tissue samples compared to that of routine staining method. The study included 30 normal mucosal samples and 10 mucocele samples. Routine processing methods were followed and two sections of each block was made. Ten pairs of sections were stained using four stains, namely hematoxylin and eosin, toluidine blue, periodic acid-Schiff and mucicarmine. One slide of each pair of section was stained by routine method and the other was stained by microwave. The results showed that no statistically significant difference observed in staining characteristics, but time consumed has reduced drastically portrays the importance of microwave assisted staining.

A critical concern for any pathology laboratory is whether Microwave assisted tissue processing method interferes with the antigenicity of the tissue. The research by Ramakrishnan et al. compared antigen retrieval in microwave-processed versus conventional tissues. Validity of ER and PR receptor status was evaluated. The sensitivity and specificity of microwave processing in ER evaluation was 87.5% and 100% while that of PR evaluation was 92.9% and 100% respectively. Microwave-assisted tissue processing offered several benefits compared to traditional methods, as it enabled quicker diagnosis, required less manual effort and produced more effective staining results making it a more favorable option.[12]

Despite the advantages, the discussion must acknowledge the limitations. Limited throughput of domestic microwave owing to its small size allows limited samples to process at a time. Thus, this makes it difficult and time consuming for processing routine biopsies in large sample sized laboratory on a regular basis. When microwave exposure occurs on reagent, release of noxious fumes occurs due to heating of reagents. Thus, to reduce the effect of noxious fumes the processing of tissue in domestic microwave was done in adequately ventilated room. The procedure is labour intensive as manual processing and staining is done in microwave which requires constant supervision and caution. Temperature should be monitored as rise above recommended level can cause detrimental effect on results of tissue outcomes. To overcome these problems large, laboratory grade microwave tissue processors with in built exhaust fans to extract toxic vapours and automated control of power, temperature and processing time would be recommended.

In view of impact and future scope of present study, microwave assisted technique shortens the tissue processing time from hours to minutes. It is responsive to the patient and physician needs, improves the use of reagents while reducing or eliminating their toxicity, creates a personnel-friendly workflow and places the laboratory in a better position to meet the demands of the rapidly expanding field of molecular medicine. Thus, Improvements in equipment design and a better understanding of tissue processing using microwave technology make the catchphrase "same-day turnaround" a reality. This quick turnaround not only reduces patient anxiety and reagent use but also increases efficiency.

Conclusion

The transition from conventional to microwave-assisted tissue processing represents a step forward in the modernization of the pathology laboratory. Microwave assisted tissue processing yielded morphology of cells and architecture of histologic material similar or superior quality to that provided by time-honored conventional processing method. Microwave method has many advantages, including expediency, safety, potential for preservation of antigenic integrity of specimens 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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