Abstract for PhD

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Study title	Comparative study of tissue processing and staining by microwave and routine method
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Purpose of study:

To meet the prerequisites of clinicians who are treating critically ill patients, it is of utmost desirable to process the histopathological biopsy specimen immediately. Conventional methods for tissue processing require 16 to 24 hours, which delays the treatment. Tissue processing by microwave method reduces the duration of cycle and permit faster processing and staining of histopathologic tissue.

By application of heat to tissue by microwave method, it enhances diffusion of fluids and dyes within the tissue. Thus it reduces the time of processing and staining. The purpose of present study is to establish microwave irradiation tissue processing and staining method that shortens the time from specimen reception to diagnosis without compromising the overall quality as well as antigenic property of tissue.

Aims and objectives:

To compare both the methods i.e. microwave and routine method for time duration of each cycle, mean tissue shrinkage, tissue quality and antigen preservation of tissue.

Review of literature:

Microwave generates heat by nonionizing radiation. The heat produced enhances the diffusion of dye molecules within the tissue which enhances rate of reaction and decreases turn around time. This makes possible to report histopathology slide earlier and clinician can start treatment in critically ill patients.

Material and method:

In present study, 350 paired tissues (total 700) were selected from histopathology laboratory of Pathology Department, S.S.G. Hospital and Medical College, Baroda. After fixation for atleast 24 hours, tissue was sectioned in twin samples and processed and stained by both microwave and routine method. Special stains and immunohistochemistry were also performed to determine antigen preservation of tissues.

In present study, Samsung microwave oven model no. MS23K3513 with maximum output of 800W was used. Tissue processing and staining were carried out with a power

output of 300 W (38% power) and 180 W(23% power) respectively. In present study, methanol, isopropyl alcohol and molten paraffin wax were used for tissue processing and staining was performed by hematoxylin and eosin stain.

Four parameters and ten subparameters were examined for result analysis. Four parameters were cellular details, cytoplasmic details, nuclear details and staining characteristics and ten subparameters were cellular outline, clarity, integrity of tissue, nuclear cytoplasmic contrast, eosinophilia /granularity, clarity of nucleus and nucleoli, clarity of nuclear membrane, clarity of chromatin, colour intensity and uniformity.

For time duration comparison, tissue processing by conventional method takes 16 hours vs microwave method takes 67 minutes. Staining of tissue also shows difference of 6 minutes i.e.14 minutes by microwave method and 20 minutes by routine method.

Results:

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 Eosinophilia/granularity and uniformity in staining by both the methods. However the difference was not statistically significant.

Various special stains like Periodic acid Schiff, Alcian blue, Massion trichrome, Fontana Massion were performed on tissues processed by both the methods and the results were comparable. The immunohistochemistry was also performed on tissues processed by both the methods and the results were comparable. Thus, antigens of tissues were preserved in microwave processing.

Summary and conclusion:

Microwave assisted tissue processing yielded morphology of cells and architecture of histologic material similar or superior quality to that provided by conventional processing method. Microwave method is rapid as it takes total 81 minutes for tissue processing and staining while routine method takes 16 hours and 20 minutes.

Microwave method 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.

Limitations of present study:

The limited throughput owing to the small size of the microwave allows limited samples to process at a time. By microwave exposure on reagent, release of noxious fumes occurs due to heating of reagents. Thus, adequately ventilated room is recommended to process tissue in domestic microwave. The procedure requires constant supervision and caution. Therefore, trained technician is required.

Impact and future scope

The technique shortens the tissue processing time from hours to minutes. The technique 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**.**