Original Article

Microwave-Assisted Histological Processing with PAS Special Stain: A Rapid Visualization Technique

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Abstract

Background: To effectuate the necessity of clinicians who treats intensely ill patients, expeditious processing of histopathologic material is becoming increasingly desirable. Using traditional techniques, expeditious processing of tissues embedded in paraffin, requires 4 to 5 hours, which would delay the treatment for intensely ill patients which also requires subsidiary shifts of specialists in the laboratory. Tissue processing by microwave method further abbreviates this time, which allows even more speedy diagnosis of histopathology. The objective is to compare the quality of the tissues executed by both conventional as well as microwave method using PAS special stain. **Material and Methods:** In this present study, 100 samples of various cell as well as tissues blocks were included which were split up into two equal halves. A half was processed by the routine conventional method and the other half lead by the recent microwave method. 10 slides (5 conventional + 5 microwave) were stained using PAS special stain and scrutinized by a self-reliant observer who was oblivious of the processing method. **Results:** There was no statistical significance recorded (p value <.545) when the tissues handled by both traditional as well as microwave techniques were compared when stained using PAS special stain. **Conclusion:** Microwave assisted processing of tissue can be endorsed for regular use in histology laboratories and considering the quality of slides produced, it would show expediency because of the shorter time taken.

Keywords: Conventional tissue processing, Microwave tissue processing, PAS special stain.

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Introduction

To effectuate the necessity of clinicians who treats intensely ill patients, expeditious processing of histopathologic material is becoming increasingly desirable. Using traditional techniques, expeditious processing of tissues embedded in paraffin, requires 4 to 5 hours, which would delay the treatment for intensely ill patients which also requires subsidiary shifts of specialists in the laboratory. Tissue processing by microwave method further abbreviates this time, which allows even more speedy diagnosis of histopathology.

In the 17th century, studies in Histology began with Marcello Malpighi. Robert Hooke described the appearance of cells in 1665. Anthony Von Leeuwenhoek observed tissues in the eighteenth century. With his task on general anatomy, Bichat, instigated the study of the tissue and the application of naked eye preparations. In the early 1700's, scientist Marie Francois Xavier Bichat introduced the study of histology and hence considered as father of descriptive anatomy and modern histology. On gross dissection, he did his task on 21 tissues mainly when compared to the use of microscopes. Previous to Bichat's discoveries, the first scientist to observe capillaries was Marcello Malpighi. He is contemplated to be the realistic "Father of Histology".[1] In the year 1909, G. Arnedt delineated the first ever automatic histo processor.[2] During the modern period, the period from renaissance to present century, numerous discoveries were made. during this time, Zacharias Jansen of Holland invented the compound microscope which opened unexplored venues of micro anatomical investigations. From there on, study of micro anatomy began and flourished as a separate branch of science.[3] Histology literally means microscopic study of cells. It is the science of tissues studied with the help of microscopes and dyes or stains. It is the colorful branch of anatomy which highlights the importance of the evaluation of the organization of cells as well as structures in the tissues.[4] Mayer found the term "Histology" in 1819. Two Greek base words "histos", which means tissues, and also logos, meaning study, were combined together. To narrate any woven substance "Histos" was used.[5] Percy Spencer discovered microwaves in the year 1945. To save time, Mayers in 1970 evaluated the theoretical prospect of utilising microwaves to accelerate the tissue processing. It was by Login, the first ever victorious reports of autopsy microwave

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fixation specimens were revealed. In processing the tissues, Kok and Boom from Netherlands in 1985, and also Anthony Leong from Australia originally appealed microwave technique.[6] In 1990s the first ever microwave histo processor was initially released to the globe by Milestone Technology.[7]

Customary histology strategies hang on slow diffusion of compounds from the external surfaces and when is put to heat, by thermal conduction, it exerts its route on the inside of the specimen. Revealing thin sections of specimens into microwave energy influences the whole specimen simultaneously and instantaneously, which enables the interchange of solutions which quickens the reaction rate owing to heat generated internally.[8] Microwaves exerts by causing spinning of charged or polar molecules. For instance, in water, one water molecule contains one atom of oxygen into which two tiny hydrogen atoms are connected.[7] Water molecules have both positive charged side and a negative charged side, so, when negative charges are brought near an electromagnetic field, there is repulsion. Since they are like charges it causes the molecules to rotate.[6] They rotate rapidly through 180 degrees at the rate of 2.45 billion cycles per second. This rotational movement produces heat.[1]

The use of microwave have been applied in many fields of histopathology and histology like histo processing, fixation, rapid staining of customary fluorescent and metallic studies and also for studies using light and electron microscopes. A frequency of 2.45GHz is designated for microwave ovens used in household since it is the frequency in which polar molecules for instance water molecules will exhibit a positive sequel and at a certain profundity the microwaves perpetuate a good robustness. This quality is a requisite for cooking food and moreover also utilised practically in histology for laboratory purpose. In contrast to laboratory oven microwave oven utilised for domestic purpose is of low-budget and replicates similar effects as that of the oven used for domestic purpose.[9]

Processing the tissues using conventional procedures is time consuming and take up a lot manpower on the other hand, the recently evolved microwave method is logical in both aspects, concerning time and manpower. It helps out in rapid fabrication of slides with negligible artifacts as well as diminution of tissue.[10]

For the study of histological light microscopy, the most customarily used stain is hematoxylin and eosin.[5] In 1908, Paul Ehrlich won the Nobel Prize also discovered the usage of methylene blue in the diagnosis of malaria and for trypanosomes he used trypan red. Countless specific stains have been made use of in the latest, for discrete kind of tissues.[11] These staining techniques being reliable and inexpensive produce preparations which lasts and are also easy to explicate and store. Hence in teaching histology various stains are exceptionally useful. Only few data prevail in comparing the quality of tissue processed with microwave and that executed by further traditional techniques utilising distinctive staining techniques. The current study was conducted to compare the quality between conventional and microwave methods in which processed tissues were stained by special stain - PAS.

Aim and objective:

To compare between conventional and microwave methods the quality of the processed tissues stained by PAS special stain.

Materials and Methods

In this present study, 100 samples of various cell as well as tissues blocks were included which were split up into two equal halves. A half was processed by the routine conventional method and the other half lead by the recent microwave method. 10 slides (5 conventional + 5 microwave) were stained using PAS special stain and scrutinized by an self-reliant observer who was oblivious of the processing method.

Inclusion Criteria

From well-embalmed cadavers, tissues were chosen randomly

Exclusion Criteria

Pathological and grossly damaged tissues were precluded.

Materials used:

1. Glass jars - 600 ml capacity
2. Cassettes used for tissue processing
3. Glass beakers (Borosil) - 500 ml
4. Glass beakers (Borosil) - 200 ml
5. Wax embedding machine
6. Tissue floatation bath
7. Rotary microtome
8. Leuckhart's embedding brass moulds
9. Paintbrushes
10. Slide warmer table
11. Egg albumin
12. Cover slips
13. Glass slides
14. Mounting media - DPX 15. Compound microscope
15. Microwave oven (Samsung): MW73AD-B/XTL 20L

Reagents used:

1. 10% formalin as fixative
2. Ethyl alcohol (Ascending concentration 50% - absolute)
3. Chloroform 100%
4. Xylene 100%
5. Paraffin wax

Stain used: Periodic acid Schiff stain (PAS).

Conventional method: Usual tissue processing procedure was done. The blocks thus prepared designated as "C," which denotes tissue processing using conventional method. Once the blocks were prepared, they were mounted on the rotary microtome for sectioning. Once they are sectioned, they were mounted onto a slide and kept on a slide warmer to remove the excess paraffin.

Microwave method: Initially, to standardize the technique a few tests were done with the reagents in the microwave. To absorb the excess heat generated while heating the tissues, the tissues were placed completely immersed in the reagent without the lid over it i.e., absolute ethyl alcohol in a 200 ml beaker and a 500 ml beaker containing water. For about 15 minutes, the tissue was passed through absolute ethyl alcohol, where the microwave was on low power mode for the first 5 minutes, and the prevailing 10 minutes on medium - low power mode. As chloroform has a high microwavability and low boiling point it was used as a clearing agent. The tissue blocks were then designated as "M" which were then sectioned with the help of rotary microtome, and thus 100 slides were poised.

Staining:

Technique of staining with PAS: After deparaffinizing to rehydrate the tissues, the slides were passed through graded series of alcohol. The slides were then placed for 5 minutes in 0.5% Periodic acid and rinsed under running tap water, rinsed again in distilled water. Then the slides were put for 15 minutes in Schiff's reagent. For a short while then put under running tap water. For 5 -7 minutes counterstained with Mayer's hematoxylin. It was then rinsed under tap water for two minutes and differentiated with acid alcohol 3 to 4 times. Subsequently for 2 minutes it was washed under tap water and then the slides were dehydrated twice in 90% alcohol for a minute each. The slides were put in absolute alcohol, dipped in xylene both for two changes of 2 minutes each and then mounted making use of DPX. The basement membrane takes up pinkish purplish stain on staining with PAS stain. 10 slides (5 conventional + 5 microwave) were subjected to staining by PAS method. Photographs were taken for documentation. After all the paired samples were processed using both conventional and microwave method of tissue processing, they were evaluated for nuclear as well as cellular morphology simultaneously by an independent observer. The slides processed with the aid of conventional method was designated as "A" and the slides processed by microwave method was designated as "B". The observer was unaware of the method of processing used during evaluation of the cellular and nuclear morphology of the slides.

Criteria for evaluation of the slides: Special staining by PAS was assessed by the basement membrane taking up pinkish purple stain adequately and inadequately. Inadequately stained slides were termed poor and adequately stained were satisfactory

Data - Collection and Recording:

1. Recording of the data was done using Microsoft Excel.
2. Statistical analysis was done using SPSS software version 22.0 and results tabulated.
3. Fisher's exact test was done to compare the quality between both the methods.
4. P value <0.05 was considered as significant.

Ethical clearance: The study was approved by the IEC.

Results

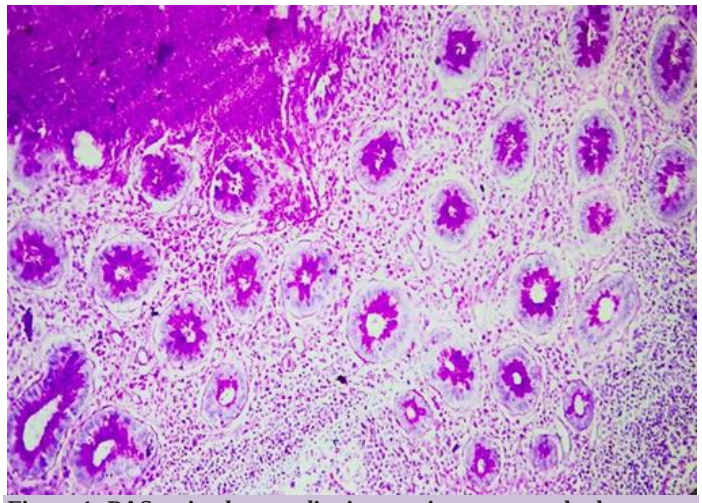

Figure 1: PAS stained appendix tissue, microwave method

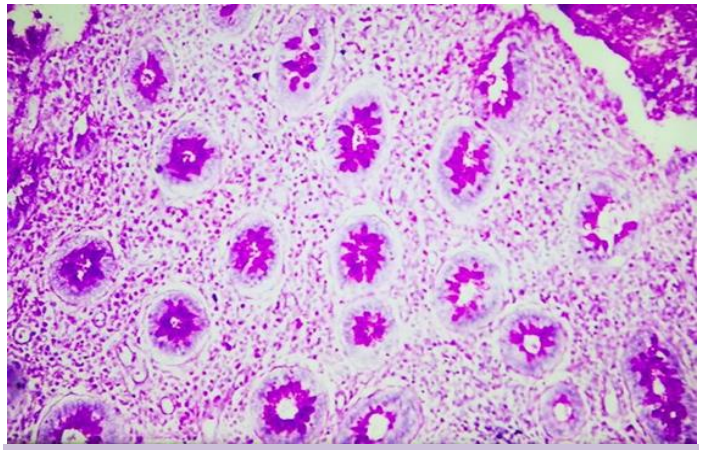

Figure 2: PAS stained appendix tissue, conventional method

Table 1: PAS staining characteristics comparison between conventional and microwave method (Fisher's exact test)

Special Stains	Quality assessment	Method		Total
		Conventional	Microwave	
PAS	Poor	1 (16.7%)	0 (0%)	1 (9.1%)
	Satisfactory	4 (83.3%)	5 (100%)	10 (90.9%)
TOTAL		5 (100%)	5 (100%)	10 (100%)

[Table 1] depicts that the staining characteristics is satisfactory in the slides processed by microwave than the slides processed conventionally. The p value was .545 which is statistically insignificant.

Discussion

Processing of tissue encompasses various strategies which includes dehydration, fixation, clearing and also wax impregnation. All these procedures are on the basis of diffusion of chemicals into the tissues.[12] In tissue processing by microwave, when the tissues fixed are treated for dehydration with absolute alcohol, the proteins encounter denaturation in the tissues so that subsidiary heat which is distributed within the microwave does not have any impact on the staining capacity of the cells. Since absolute alcohol behaves as a coagulant fixative as well, it preserves the architecture of tissue.[13]

Microwaves are non-ionizing in nature and electromagnetic waves, which has the capability to pierce wide variety of materials. The electric conductivity of medium determines the depth of penetration. Owing to the microwave irradiation reaction oscillating electric fields are brought out where

molecules such as water will vibrate. During vibration the acquired rotational energy will be transferred to random motion during collision with other molecules of water. This sort of kinetic movement produces heat inside the tissues. The heat thus produced will sequentially increase the reagents' diffusion rate inside the tissues and hence reduces the time taken for processing the tissue.[14]

Scientists and researchers have done countless studies to bring to light new and expeditious methods of processing tissues to enable results on the same day. Hence, the microwave method of processing tissues formulated was time saving and also produces slides comparable with negligible differences when it was compared with 55 conventional methods. In this context, one of the ancient studies was done in 1985 by Anthony Leong as well as Kok and Boon in 1986.[15,16]

In the year 1893, formalin was introduced. In the 17th century scientists like Leeuwenhoek devised techniques from which the early staining techniques came to be. One of the oldest stains to be used for study was Prussian blue. Wissowzky described the hematoxylin and eosin staining techniques in 1875. Periodic acid was introduced in 1928 by Malprade for the chemical assessment of polyalcohols. In histology McManus first applied in 1946 the periodic acid Schiff-reaction.[16] In 1937 It was employed by Jackson and Hudson as a reagent for polysaccharide as well as glycogen and was demonstrated by Schabdacsh in 1947.[17] In 1948 it was overworked by Lillie soon after was used for illustrating a wide range of mucosubstances in tissues. Principle of periodic acid lies in the breakage of C-C bonds of numerous structures when they are seen in form of 1:2 glycol groups converting them into aldehydes. These aldehydes recolorise the colorless Schiff reagent and the locations of PAS reactive groupings are indicated by magenta or red color. This specific trait of PAS manifests it measurelessly superior to other reagents which are used in histochemistry for the oxidation of the C-C bands and preclusion of its conversion into carboxyl group which makes it an exceptional stain in routine histochemistry.[17,18]

Periodic acid-Schiff (P.A.S.) technique, developed by Manus, Lillie and Hotchkiss, is more often believed to demonstrate the existence of I :2 glycol (and a-aminoalcohol) groups in tissues. It is presumed that these groupings are oxidized by periodic acid into aldehyde groups which then react with fuchsin decolorized with sulphurous acid (Schiff reagent) to give purple- red compounds.[19]

In the present study, there was no statistical significance. This is similar to the findings in the study done by Godwin where the staining quality was better in the case of microwave processed tissues than the conventionally processed tissues.[20] On the other hand, the quality of the special staining done by PAS was found to be better in the sections obtained from tissues processed by microwave method than the conventional method.

Conclusion

The histological quality in the new microwave method was better when compared to the usual manual technique. Tissue processing with the assistance of microwave can replace the gold standard of routine tissue processing considering the time taken and the exceptional quality of the histological slides produced by special staining techniques. It would also enable technical personnel by eliminating the constant use of xylene during tissue processing.

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Conflicts of interest

There are no conflicts of interest.

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