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Original Research Article

Tissue Processing using Microwave Oven: A Boon for Histology Slide Preparation

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ABSTRACT

Background: Tissue processing is an important step in histology laboratories. Routine tissue processing is time-consuming. Due to the same time factor, microwave tissue processing is now increasingly being used in many histology and pathology laboratories.

Objective: To compare the time taken to process the tissues using the routine conventional and microwave oven methods.

Methods: The study was conducted in the Histology laboratory. 100 slides (50 conventional method + 50 microwave method) were processed using both methods, sectioned, stained, and analyzed by an independent observer who was unaware of the processing method.

Results: The study showed a significant decrease in processing time by the microwave method (p-value < .0001).

Conclusion: The present study concluded that the time taken for dehydrating, clearing, and embedding the tissues in paraffin wax was found to be considerably lower than that taken for the conventional method of tissue processing. Chloroform was used here as a clearing agent, and it had desirable effects.

KEYWORDS: Microwave oven tissue processing, Conventional tissue processing.

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INTRODUCTION

Histology is the study of the microscopic anatomy of cells and tissues of plants and animals. It is usually performed by examining the cells and tissues under a light microscope after the preferred specimen has been sectioned, stained, and mounted on a glass slide. Marie Francois Xavier Bichat was French anatomist and pathologist was considered as the Father of Animal Histology. He introduced the definition of tissue [1].

Tissue processing is the process of preparing the tissue by embedding it in a solid medium that is firm enough to support it and make it rigid enough to section it according to a desirable thickness. In the meantime, the tissue is also retained soft enough for the knife to cut through it without damaging

it. Processing the tissue enables easy visualization of the tissue under the microscope to study the characteristic features of the various organelles within the cell [2].

Tissue processing is a complex procedure that involves various steps. It is done to remove the water content from the tissues and replace it with a medium that hardens to allow thin sections to be cut, ideally 5 micrometers in thickness. The sample tissues are first transferred through baths of progressively concentrated ethanol to remove the water. This is followed by a clearing agent such as xylene or chloroform to remove the alcohol and finally passed through molten paraffin wax, which is used as an infiltration agent to replace xylene, after which the tissues are ready for external embedding [2].

The tissues are placed in moulds along with liquid paraffin wax, which is then hardened by cooling the paraffin wax. The hardened blocks containing the tissue samples are sectioned using a microtome and stained with hematoxylin and eosin stains. They are mounted on a glass slide and viewed through the light microscope.

Tissue processing is important in obtaining good thin sections without many artifacts. G.Arnedt described the first automated histological processor in 1909 [3]. From 1910 onwards, the main techniques for histological processing have been set out and are being followed to date. Percy Spencer discovered microwaves in 1945. In 1970, Mayers tested the theoretical possibility of using microwaves to speed up tissue processing to save time. The first successful reports of microwave fixation of autopsy specimens were reported by Login. In 1985, Kok and Boom from the Netherlands and Anthony Leong from Australia first applied the microwave technique in processing tissues [4,5]. Milestone Technology was released as the first microwave histological processor in the 1990s [5].

The time taken for dehydrating, clearing, and embedding the tissues in paraffin wax was found to be considerably lower than that taken for the conventional method of tissue processing. Chloroform was used here as a clearing agent, and it had desirable effects.

Routine histology procedures depend on the slow diffusion of solutions from the outer surfaces. If heat is applied, it works its way into the specimen's interior by thermal conduction. Exposing thin sections of specimens to microwave energy affects the entire specimen instantaneously and simultaneously, enabling the exchange of solutions and speeding up the reaction rate due to the internal heat generated [5]. Microwaves work by causing the rotation of polar or charged molecules. For example, in water, one molecule has one atom of oxygen to which two little hydrogen atoms are attached [2-4].

Water molecules have both positively and negatively charged side, so when negative charges are brought near an electromagnetic field, there is repulsion. Since they are like charges, the molecules rotate [3]. They rotate rapidly through 180 degrees at the rate of 2.45 billion cycles per second. This rotational movement produces heat [1].

Materials that heat up fast are composed of nonsymmetric polar molecules and are rotated easily by microwave energy [5]. This acquired rotational energy is passed into random motion with a collision with other molecules. When the molecules collide, it absorbs the microwaves and converts them into kinetic and thermal energy. Heating in the microwave is internal and affects the material when irradiated [2-4].

Microwave use has been employed in many fields of histology and histopathology such as fixation, histological processing, rapid staining of routine, metallic and fluorescent studies, and both light and electron microscopic studies. A frequency of 2.45GHz is selected for household microwave ovens because it is the frequency at which polar molecules, like water, will show a positive outcome, and the microwaves maintain good strength at a certain depth. This property is essential for cooking food and is used practically in histology for laboratory work. A domestic microwave oven is economical compared to the laboratory oven and reproduces similar results to that of the domestic oven [6].

Conventional methods of tissue processing take up a lot of time and manpower, while the newly evolved microwave method is efficient in both aspects, regarding time and manpower. It helps rapidly to produce slides with minimal artifacts and tissue shrinkage.

This study is aimed to compare the time taken between conventional method and microwave methods of tissue processing.

METHODS

A comparative study was conducted in the Histology laboratory. A sample of 50 paired slides (50 conventional method + 50 microwave) was prepared with a thickness of 1cm x 1cm, about 5 to 8mm without any bony component.

Inclusion Criteria: Tissues were randomly chosen from well-embalmed cadavers

Exclusion Criteria: Grossly damaged and pathological tissues were excluded.

Materials used:

· 600 ml glass jars
· Tissue processing cassettes
· Borosil glass beakers 500 ml
· Borosil glass beakers 200 ml
· Wax embedding machine
· Tissue floatation bath
· Leuckhart's embedding brass moulds
· Rotary microtome
· Paint brushes
· Egg albumin
· Slide warmer table
· Glass slides
· Cover slips
· Mounting media
· Compound microscope
· Samsung microwave oven- voltage-230V, AC power input 1200W, power output 800W, Frequency 2450 Mhz.

Reagents used:

· 10% formalin as fixative
· 50%, 70%, 90% ethyl alcohol and absolute ethyl alcohol
· 100% Xylene
· 100% Chloroform
· Paraffin wax

Stains used: Hematoxylin and Eosin stains

The tissue specimens were all fixed in 10% formalin.

The tissue specimens selected were:

· Large artery
· Vermiform appendix
· Lung
· Liver
· Kidney
· Testis
· Skeletal muscle.

Only tissues without bony components were selected for this study. The specimens were about 1cm x 1cm and about 5 to 8 mm thick. The tissue specimens were equally divided into two halves; one half was processed by the conventional method, and the other half was processed by the microwave method.

Conventional method: The procedure began in the morning on the first day. The tissue cassette containing the specimen was placed in water for an hour's time to wash off excess formalin. After which, it was placed into glass jars containing ascending grades of ethyl alcohol, beginning with 50% ethyl alcohol, then 70% ethyl alcohol, then 90 % ethyl alcohol, and finally into absolute alcohol for half an hour each. This is the step of dehydration, where the water content in the tissues is removed by passing them through serial gradations of alcohol. Then it was passed through two cycles of 100% xylene for half an hour each to clear the tissues. This is the step of clearing where the tissues are made miscible with paraffin wax.

The next day, the tissue cassettes were passed through the wax embedding machine containing molten paraffin wax for three cycles, each lasting about half an hour. Then, the blocks were prepared using L-moulds and labeled as "C," which is the conventional tissue processing method. Once the blocks were prepared, they were mounted on the rotary microtome for sectioning. Once sectioned, they were mounted onto a slide and kept on a slide warmer to remove the excess paraffin.

Microwave method: Initially, a few tests were done with the reagents in the microwave to

standardize the technique. The reagents and a beaker of water were kept in the microwave after placing the tissues in water for an hour to remove the excess formalin.

Standardization of technique was done as follows: The tissues were placed into beakers with the reagent and heated at 5 minutes, 10 minutes, and 20 minutes. The tissues heated for 5 and 10 minutes showed inadequate dehydration and clearing. The tissues treated at 20 minutes were difficult to work with. Meanwhile, 15 minutes of oven treatment showed optimum dehydration and clearing. Hence, 15 minutes was chosen as the optimum time for this study. This is in accordance with the study by Klump et al [4] and Kango and Deshmukh [13].

The temperature was measured with the thermometer and was kept in between 45 to 58 degrees centigrade [3].

Technique: The tissues were placed completely immersed in the reagent without the lid on, i.e., absolute ethyl alcohol in a 200 ml beaker and a 500 ml beaker containing water. This was done to absorb the excess heat generated while heating the tissues. The tissue was passed through absolute ethyl alcohol for about 15 minutes, where for the first 5 minutes, the microwave was on low power mode, and the remaining 10 minutes were on medium-low power mode.

The same procedure was used for clearing with 100% chloroform, with the first five minutes on low power mode and the remaining 10 minutes on medium-low power mode. Chloroform is a clearing agent because it has a low boiling point and high microwavablity. Then, the tissue was embedded with paraffin wax, with the first 5 minutes on medium-low mode and the next 10 minutes on low mode. This was done to remove all the chloroform from the tissue while treating it with paraffin wax.

The tissues were then embedded in wax using the L-moulds and left to harden. These blocks were labeled "M." Then, the blocks labeled C and M were sectioned using the rotary microtome, and 100 slides were prepared.

Staining: The routine hematoxylin and eosin (H&E) procedure was used to stain all 100 slides equally.

The procedure followed was as follows:

The slides were deparaffinized by gently warming them over the slide warmer table for a minute each and then placed into a tray filled with 100% xylene. Two consecutive changes of xylene were done for 2 minutes each. The slides were passed through a graded series of alcohol to rehydrate the tissues. Beginning with absolute alcohol for 3 minutes, followed by 70% ethyl alcohol for 2 minutes, followed by 50% ethyl alcohol for 2 minutes. It was then dipped into the tray containing Mayer's hematoxylin for 5 minutes and placed in a water bath for 2 minutes. The slides were viewed under the microscope to determine excessive bluish staining. Then, the slides were placed in 1% acid alcohol for a few seconds. The bluish color was regained by washing under alkaline tap water. Then, the slides were placed in the tray containing 1% aqueous eosin for 30 seconds to 1 minute. The stained slides were dehydrated again following a graded series of alcohol beginning with 50% ethyl alcohol, followed by 70% ethyl alcohol, followed by 90% alcohol for a minute each. Then, the slides were placed in two changes of absolute alcohol for two minutes each. The slides were then dipped in xylene for two changes for 2 minutes each and then mounted with a resin mountant containing discrete dibutyl phthalate and xylene. It's commonly known as DPX.

Hematoxylin stains the nucleus blue, and eosin stains the cytoplasm pink.

All 100 slides (50 conventional method + 50 microwave method) were subjected to H&E staining.

Collection and Recording of Data:

The data was recorded using Microsoft Excel.

Statistical analysis was done using SPSS software version 22.0, and results were tabulated.

Fisher's exact test was done to compare the time taken between both methods.

A p-value of <0.05 was considered statistically significant.

Ethical clearance: The Institution Ethics Committee approved the study.

RESULTS

Table 1: The comparison between the time taken for dehydration by conventional and microwave method (Fisher's exact test)

		Method		Total
		Conventional	Microwave	
Dehydration (Mins)	15	0	50	50
		0%	100.00%	50.00%
	120	50	0	50
		100.00%	0%	%
Total		50	50	100
		100.00%	100.00%	100.00%

Table 1 shows that in the microwave method, the time taken for dehydration is 15 minutes which is significantly lower when compared to 120 minutes taken by the conventional method. The p value was found to be .0001 which is significant.

Table 2: The comparison between the time taken for clearing by conventional and microwave method (Fisher's exact test).

		Method		Total
		Conventional	Microwave	
Clearing (Mins)	15	0	50	50
		0%	100.00%	100.00%
	60	50	0	50
		100.00%	0%	100.00%
Total		50	50	100
		100.00%	100.00%	100.00%

Table 2 shows that in the microwave method, the time taken for clearing is 15 minutes which is significantly lower when compared to 60 minutes taken by the conventional method. The p value was found to be .0001 which is significant.

Table 3: The comparison between the time taken for wax impregnation by conventional and microwave method (Fisher's exact test)

		METHOD		TOTAL
		CONVENTIONAL	MICROWAVE	
Wax Impregnation (Mins)	15	0	50	50
		0%	100.00%	100.00%
	90	50	0	50
		100.00%	0%	100.00%
Total		50	50	100
		100.00%	100.00%	100.00%

Table 3 shows that in the microwave method, the time taken for wax impregnation is 15 minutes which is significantly lower when compared to 60 minutes taken by the conventional method. The p value was found to be .0001 which is significant.

DISCUSSION

Tissue processing comprises various procedures, which include fixation, dehydration, clearing, and wax impregnation. All these steps are based on the diffusion of the chemicals into the tissues [1]. Fixation is the first step in histological tissue processing. This step was introduced by Blum in the year 1893. By this process, the cells within the tissue are preserved as such, enabling us to learn more about the structure of the cells in the tissue. It is usually done by leaving the desired tissues completely immersed in 10% formalin for a period of 6 to 24 hours. The advantage of fixing the tissues in formalin is that it enables hematoxylin staining and produces minimal shrinkage of the tissues. Apart from being toxic to humans, formalin is also known to cause profound hardening of the tissues at times [2].

Dehydration is the next procedure, where the tissue is passed through varying ascending strengths of alcohol to remove the free water within the cells. This step enables the tissue to be miscible with the paraffin wax while embedding during block preparation and also helps the tissue to mount properly while mounting with Canada Balsam. The procedure ideally begins with 30% ethyl alcohol and progresses to 50%, 70%, and 90% and ends with two changes of absolute alcohol. This step ensures all the water has been removed from the cells. Commonly used dehydrating agents are alcohol and acetone [2].

The tissues are placed in a clearing agent to remove all the alcohol from the tissues. This step is also called de-alcoholization. It makes the tissue clearer while viewing under the microscope, and it should also be miscible with paraffin wax during the wax impregnation process. In the conventional tissue processing method xylene is ideally used as a clearing agent. However, in the case of microwave tissue processing, xylene cannot be used due to its low microwavability. Hence, chloroform was used instead. Then comes the stage of wax impregnation, whereby the tissues are embedded in a medium that replaces the clearing agent from within the tissue and

makes the tissue strong enough to withstand the trauma of sectioning and thereby provide support [2].

In microwave tissue processing, when the fixed tissues are treated with absolute alcohol for dehydration, the proteins in the tissues undergo denaturation, so additional heat which is delivered within the microwave, doesn't affect the staining capability of the cells. Absolute alcohol also acts as a coagulant fixative, hence preserving the tissue architecture [3].

Other studies have used varying alcohol strengths to dehydrate the tissue during microwave histological processing. In a study by Kumar et al. [7], the tissues were exposed to 80% ethyl alcohol for 10 minutes, followed by absolute alcohol for two changes of 10 minutes each. However, in the current study, the tissues were directly exposed to absolute alcohol for 15 minutes without pre-exposure to any reduced strengths of alcohol, and desirable results were achieved.

Microwaves are electromagnetic waves, non-ionizing in nature, which can penetrate different kinds of materials. The depth of penetration depends upon the electric conductivity of the medium. Due to the microwave irradiation effect, electric fields are produced where molecules like water will vibrate. During this vibration, the rotational energy acquired will be transferred to random motion during collision with other water molecules. So, this kind of kinetic movement builds up heat within the tissues. This heat produced will, in turn, increase the diffusion rate of the reagents into the tissues, there by reducing the time taken for tissue processing [8].

Various studies have been done by researchers and scientists to discover new and rapid methods of tissue processing to enable same-day results. Thus, the microwave method of tissue processing was devised to save time and produce comparable slides with minimal differences compared to conventional methods. One of the oldest studies done in this regard is by Anthony Leong in 1985 and Kok and Boon in 1986 [4].

In the present study, out of the 50 paired slides prepared, it was recorded that the time taken for dehydration, clearing, and wax impregnation was 45 minutes, which was less than an hour. This time is in accordance with the studies done by Klump et al. [4] and Kango and Deshmukh [13]. Von Seebach studied graded ethanol, xylene, and paraffin wax at a temperature of 75ºC, where the time taken was 111 minutes.

The time taken for clearing using chloroform in this study was 15 minutes, which is similar to the study done by Klump et al. [4] and Kango and Deshmukh[13]. On the contrary, in the study by Kumar et al. [14], the time taken for clearing was reduced to 10 minutes and was found to yield good and clear sections. But in the present study, it wasn't favourable for us when we reduced the time taken for clearing; the sections hadn't taken up adequate stain indicating inadequate clearing.

Various studies have used the same technique, but isopropanol and isopropyl alcohol at a greater temperature as an intermedium after dehydration is used to remove the alcohol from the tissues effectively before wax impregnation. The time taken for wax impregnation was 15 minutes in the present study, which is similar to the studies done by Klump et al. [4] and Kango and Deshmukh [13].

There are other studies where the time taken for wax impregnation was reduced, and it was found to have obtained good sections with distinct cellular features like in the studies by Kumar et al. [14].

The overall time taken for the present study, excluding fixation by formalin, sectioning, and staining, was less than an hour, 45 minutes to be exact. This is in accordance with the studies done by Klump et al. [5] and Kango and Deshmukh [13]. In the present study, the time taken was comparatively less when compared to other studies by Kumar et al. [14], Morales et al. [15], Leong et al. [16], and others.

CONCLUSION

Microwaves are non-ionizing electromagnetic waves that, when applied in histology laboratories for histological techniques, yield reproducible histologic slides of similar and

superior quality to traditional and conventional gold-standard tissue processing methods. Domestic microwave ovens are advantageous mainly because they use less time, are compact in size, preserve the specimen well, and do not hamper the laboratory workflow, which permits rapid preparation of the histologic material.

The time taken for dehydrating, clearing, and embedding the tissues in paraffin wax was found to be considerably lower than that taken for the conventional method of tissue processing. Chloroform was used here as a clearing agent, and it had desirable effects.

Author Contributions

Dane Chandy- Designed and conducted the study. **Prima Swetha Dsouza:** Manuscript writing and analysis.

Susie J David: Interpretation and statistical analysis.

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Conflicts of Interests: None

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