ORIGINAL ARTICLE

A comparative study of microwave oven-assisted tissue processing and conventional method of tissue processing on turnaround laboratory time and morphological quality of tissue sections

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ABSTRACT

Introduction: The purpose of tissue processing is to fix the tissue in a solid medium toenable thin sections. Conventional method of tissue processing is the standardized method of tissue processing which has been used for more than 10 decades. However, the conventional method is time-consuming, and the overall turnaround time for the histopathology report is at least two days. The objective of this study is to identify the protocol for tissue processing procedure using domestic microwave oven. To determine the tissue processing time when using domestic microwave oven. To compare the morphological quality of tissue slides made by domestic microwave oven and conventional method using automated tissue processor.

Matrials and Methods: The conventional protocol and three microwave protocols of tissue processing were used in this study. A pilot study was done prior to the real run to determine the baseline timing for microwave protocol. The baseline timing was fixed at 2 minutes,30 minutes,5 minutes and 25 minutes. The processing time of the microwave protocol was adjusted from 62 minutes to 70 minutes to 77 minutes by increasing the dehydration and wax impregnation time while the time for tissue fixation and clearing remain the same throughout all the microwave protocols.

Results: The group 2 microwave protocol produced the sections that is closely comparable to group 1 conventional protocol. The morphological quality of histopathology slides is best observed when the processing time of microwave protocol is 62 minutes.

Conclusion: The most appropriate microwave protocol for tissue processing is group 2 as the morphological quality of histopathology slides are more superior than that of group 1 with an overall percentage of 80% of satisfactory slides in group 2 and 76.68% in group 1.

KEYWORDS:
Tissue processing, microwave, histopathology, morphological quality

INTRODUCTION

A histopathology slide that is viewed under microscope by pathologist is produced through tissue processing in the histology laboratory for diagnosis.[1] Tissue processing is a procedure that needs to take place between tissue fixation and the sectioning or embedding of paraffin blocks and it comprises of four steps which are dehydration, clearing and impregnation. Tissue processing is very important because when the tissue samples are not properly processed, there might be difficulty in sectioning the tissue samples, and therefore the microscopic information produced will not be helpful.[2]

It is undeniable that tissue biopsy and diagnosis areindeed important for appropriate patient management and choice of therapy. To get the tissue diagnosis from histopathologist, the tissue must first be processed. In routine histopathology laboratory, the tissue samples are processed by automated tissue processor. This conventional procedure has been standardised and used for more than ten decades. Thus, conventional tissue fixation and processing remain as the gold standard against all new technologies and methods.[3] However, routine processing requires many steps and take time, which can delay the diagnosis and management of the patient and lead to serious consequences. It takes approximately 12 hours for tissue to be processed in automated tissue processor machine, and therefore the overall turnaroundtime for the report of tissue biopsy by a histopathology laboratory is at least 2 days.

Microwave ovens arenow used widely in laboratory. For example, microwave oven is used in the laboratory for drying glassware, regeneration of drying material and activation of thin-layer chromatography plates. Besides that, microwave ovens have also become increasingly popular for use in tissue processing and is found to be useful for tissue processing in a short time.[4]

MATERIALS AND METHODS

This study was done at the research laboratory, International Medical University, Malaysia during the period of April 2018 – September 2018. The study samples included soft tissues and visceral organs which were randomly selected from the

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university's animal house. Hard tissue samples such as bone tissues were excluded from the study.

Tissue Processing with Conventional Tissue Processor
Tissue samples were placed in plastic cassettes and processed using Leica automatic tissue processor on an overnight programme from formalin (10%, 1 hour 30 mins), through graded ethanol (50%, 1 hour 30 mins; 70%, 1 hour 30 mins, 95%, 1 hour 30 mins, 95%, 1 hour 30 mins, 100%, 1 hour 30 mins) to xylene (2 buckets, 1 hour 30 mins each) to molten paraffin wax (2 buckets, 1 hour 30 mins each).

Tissue Processing with Domestic Microwave Oven
A domestic microwave oven (Sharp microwave oven, modelno: R207EK, powersource: 230- 240V, 50Hz, outputpower: 900W) was used in our study.The four glass beakers were filled with solutions, respectively, prior to processing the tissue in the microwave oven. Although the tissue samples were fixed in formalin at room temperature prior to the day of tissue processing, the tissue samples were still microwaved for two minutes in 10% formalin to make sure that the tissues were fixed adequately. The samples were then microwaved with a mixture consisting of equal quantities of 2-propranolol and acetone for dehydration. Xylene is used for the clearing process, and this is followed by waximpregnation.

A pilot study with 30 samples wascarried out to standardise the baseline timing for the procedure. The baseline timings were fixed at 2, 30, 5 and 25 minutes for tissue fixation, dehydration, clearing and wax impregnation, respectively.

The temperature of the solution is measured after each tissue processing step, and the microwave oven was left to cool for a couple of minutes before proceeding to the next step of the processing. A beaker containing an equal amount of distilled water was placed in the microwave throughout the four tissue processing steps to prevent overheating of the solution. For every step of tissue processing, fresh solutions were used.

All the reagents were heated directly in the domestic microwave oven except for the paraffin wax. The paraffin was melted separately on a hot plate prior to the wax impregnation processing step.

Table I depicts the protocols A, B and C with the time allotted (in minutes) of each stage of tissue processing accordingly.

Methods of Evaluation of Processed Slides
All the slides were evaluated by two experienced histopathologist without prior knowledge to which techniques were used to process the tissue samples. The morphological qualities of microscopic slides were analysed using light microscopy and a score of 1 (satisfactory) or 0 (unsatisfactory) were given to the slides. The parameters used for evaluation were the cytoplasm, nucleus morphology and staining characteristics. The slide was graded satisfactory if two or three parameters score 1 whereas it was graded unsatisfactory if none or only one of the parameters scored1. Table II provides the histological parameters along with its features to be graded for the morphological analysis.

RESULTS

A total of 80 tissue samples were processed in this study by both conventional method and three different microwave method. The tissue samples were divided into four groups equally, with 20 tissue samples per group. This is because previous literature such as Devi et al concluded that the morphological quality of tissue samples processed by domestic microwave oven wascomparable to tissue samples processed by a conventional method when the sample load in microwave oven was up to 25 samples.[5] In this study, 10% formalin is used for tissue fixation, propranolol with acetone is used for dehydration, xylene is used for clearing and paraffin wax is used for wax impregnation.

Muscular tissues which include both skeletal muscle and heart muscle tissue constituted to the highest percentage of tissues, together making 51.25% of the total samples and have the overall highest percentage of satisfactory slide among all the types oftissue.

The percentage of satisfactory slides of group 2 microwave protocol (Protocol A) is closely comparable to group 1 conventional group, as group 2 has an overall percentage of 80% where as group 1 has an overall percentage of 76.68%. Therefore, Group 2 microwave protocol is more suitable for tissue processing as compared to group 3 (Protocol B) and 4 (Protocol C). The morphological quality of tissue slides is best observed for microwave tissue processing protocol of group 2 with the tissue processing time of 62 minutes which is much better than group 3 and 4 microwave protocols, which have a tissue processing time of 70 min and 77 mins respectively and way better than group 1 conventional method, which has a tissue processing time of 18 hours. Where there's a difference in opinion, the two pathologists discuss and reach a consensus on the difference of opinion cases.

The morphological quality of histopathology slides of microwave protocol group 2 is superior as compared to group 1 (conventional method). This is shown through the average percentage of satisfactory slides, group 2 has a higher percentage compared to group 1. However, the morphological quality of histopathology slides of microwave protocol groups 3 and 4 is inferior as compared to group 1 (conventional method).

Groups 3 and 4 has 63.88% and 38.88% of slides that are graded as satisfactory and this is significantly lower compared to groups 1 and 2 which has 76.68% and 80% of satisfactory slides. The lower percentages of satisfactory slides in groups 3 and 4 is because the tissue samples processed in these groups showed a lot of degenerative changes. Among all the types of tissue processed, muscular tissues show much consistent results in all protocols whereas liver and spleen tissues show maximum degeneration in comparison to other tissues. Table III provides the summary and comaprision of morphological quality of the histopathology slides among the groups 1 to 4.

DISCUSSION

The total time for microwave tissue processing was increased gradually from 62 minutes to 70 minutes and 77 minutes.

Table I: Protocol for domestic microwave tissue processing

Microwave tissue processing (minutes)	Fixation-10% formalin	Dehydration-propanolol+ Acetone	Clearing-xylene	Wax impregnation
Protocol A	2	30	5	25
Protocol B	2	35	8	25
Protocol C	2	40	10	25

TableII: Rubrics for qualitative morphological analysis of histopathology slides [Devi et al][5]

Parameters	Features
Cytoplasm	Nuclear—cytoplasmic contrast; Eosinophilia of cytoplasm
Nucleus	Nuclear membrane; chromatin condensation; mitotic figures
Staining characteristics	Eosinophilic cytoplasm, nuclear cytoplasmic contrast, crisp staining of nucleus,

Table III: Comparison of percentage of satisfactory slides for each group

Type of tissue	Group 1 (Control)	Group 2	Group 3	Group 4
Liver	60%	0%	33.3%	0%
Kidney	66.7%	100%	50%	33.3%
Lung	66.7%	N/A	50%	50%
Skeletal Muscle	66.7%	100%	100%	100%
Heart	100%	100%	100%	50%
Spleen	100%	100%	50%	0%
Average %	76.68%	80%	63.88%	38.88%
Turnaround time	18 hours	62 mins	70 mins	77 mins

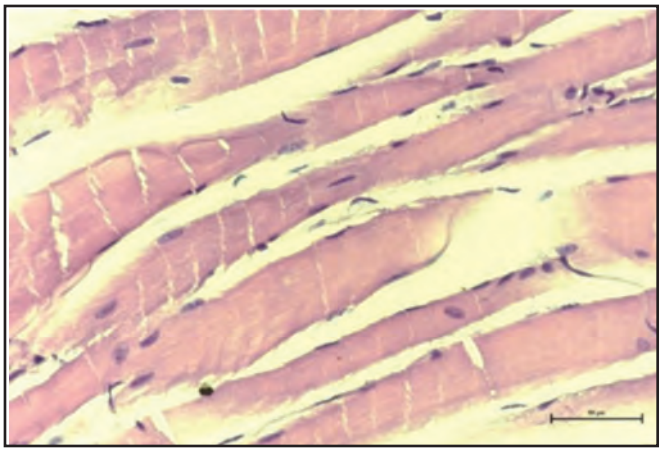

Fig. 1: Section of skeletal muscle from group 2

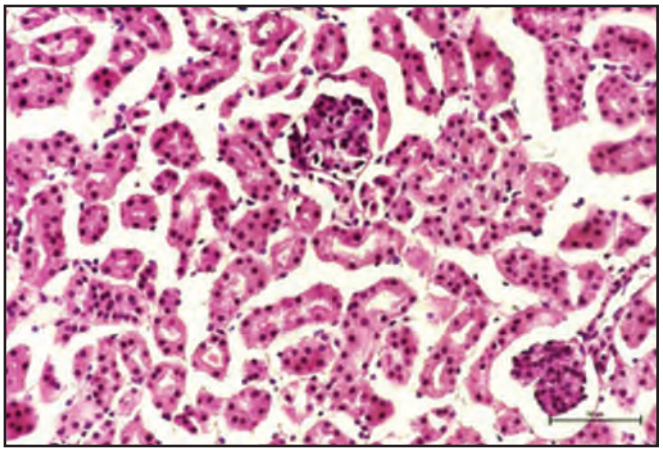

Fig. 2: Section of kidney from group 4

Tissue processing time in group 2 is 62 minutes which is much better than group 3 (70 minutes) and 4 (77 minutes), and way better than group 1, which has a tissue processing time of 18 hours. Group 2 also has the highest average percentage of satisfactory slides, therefore morphological quality of tissue slides is best observed when tissue processing time is 62 minutes.

The microwave power for fixation, dehydration and clearing tissue processing steps is fixed at 40 power for our domestic microwave oven used, whereas the microwave protocol for wax impregnation is fixed at 30 power for all microwave protocols. The microwave power is decided to be fixed at these powers through pilot study. When the microwave power is fixed at a power higher than 40, tissue is charred; however, if the microwave power is fixed at a power lower than 30, tissue is not able to process properly.

Groups 3 and 4 have a lower percentage of satisfactory slides as compared to groups 1 and 2 due to the degenerative changes shownby the tissue samples. The reason for tissue degeneration could be due to several factors. Firstly, the tissue samples came from a diverse sample group. These leftover animal carcases are used by other researchers prior to this research project. The conditions that the animals have gone through before sacrificing might be different with each animal carcase. Besides that, the duration that the animal carcase was left at room temperature before storage in freezer was unknown and time interval that the carcase stored in the freezer at the animal house facility was not able to be control as well. The microanatomy of the organs could also be one of the factors affecting the quality of slides. Liver and spleen tissues are highly vascular and have less connective tissue thus degeneration sets in faster if they were not properly stored in freezer or immediately fixed in formalin. Another

reason affecting the morphological quality of tissue slides could be due to the change in duration between protocols causing a variation in temperature when processing the tissue samples.

There were difficulties in taking paired tissue samples as the size of each animal were very small and it's quite impossible to divide the tissue samples into 4 equal sizes especially for the heart tissue and lung tissue. Besides that, the propranolol and acetone evaporated very quickly and aggressively during the microwave tissue processing process. As a result, the reagent had to be top up in the middle of the dehydration step to make sure all the cassettes are fully immersed in the solutions throughout the duration for properdehydration.

Gross sectioning is done in such a way that all the tissues samples obtained are of a similar size. This is because tissue samples of a larger size will need a longer time to be processed as compared to the smaller size tissue samples, and this will cause the smaller size tissue samples to be charred. Therefore, having a similar size of tissue samples will prevent any interference of the optimisation of the microwave tissue processing protocol.[5]

During the pilot study, the tissues were processed with a shorter duration for dehydration and wax impregnation. However, there is difficulty in cutting the tissues into thin sections as the tissues samples are not processed thoroughly and tissue samples are not properly dehydrated. In the initial part of trial study, the dehydration step of tissues is processed through only propranolol, which are the technique used by Rohretalin 2001; however, we noticed that the morphological quality of tissue slides processed through equal mixture of propranolol and acetone have a higher percentage of satisfactory score as compared to tissue slides processed through only propranolol.[6] Hence, we have decided to use both propranolol and acetone through out our microwave tissue processing protocol.We also noticed that atleast 25 minutes is needed for wax impregnation, which is slightly shorter compared to Devi et al, but slightly longer when compared to Kango et al, where he reported that the time for wax impregnation ranges from 5 minutes to 15 minutes.[5,7]

There was also difficulty in fixing the temperature of the microwave oven at one fix temperature as the domestic microwave oven that was used had limited control. Consequently, the temperature of there agent is measured manually at the end of each processing step, and this temperature is recorded throughout all theprotocols.

Our study has an overall tissue processing time of about 60 minutes (excluding tissue fixation, sectioning and staining of slides) when using domestic microwave oven for tissue processing. This is consistent with studies done by Panja et al, Kumar et al and Rohr et al and slightly longer when compared to the study done by Bond et al which has a microwave protocol of 42 minutes.[6,8,9,10]

The morphological quality of histopathology slides of tissues processed by automated tissue processor and domestic microwave oven in our study are very similar, with group 2 microwave protocol histopathology slides having as light superiority of quality as compared to group 1 conventional protocol. Likewise, Rohr etal and Bond etal also reported that the quality of histopathology slides of tissues processed by microwave protocol are more superior or similar to the tissues processed by conventional protocol.[6,10]

The same chemicals were used in our study and with study done by Devi et al in 2013 which was by using equal mixture of propranolol and acetone for dehydration and xylene for clearing instead of chloroform. However, Devi etal have a longer microwave tissue processing time (1 hour 46 minutes for a load of 20 samples) compared to our study. Nevertheless, both study has a similar result in the percentage of satisfactory slides.[5]

As a comparison between our research with other researchers from the table, we can see that the percentage of satisfactory slide of group 2 microwave oven tissue processing protocol is similar with other researchers such as Devi et al, Kumar et al and Kango et al in such a way that the percentage of satisfactory slides of tissue processed by domestic microwave oven is higher than that of tissue processed by automated tissue processor.[5,7,9] The reason why the result of our study is similar to these studies could be due to several reasons.

However, in the study done by Rohr et al, the percentage of satisfactory slide of conventional method is slightly higher than microwave method. This is also similar in our study when we compared the percentage of satisfactory slide of group 3 microwave protocol, which has a percentage of around 64% to group 1 conventional protocol, which has a satisfactory percentage of around 77%. In the study done by Rohr, he reported that the unsatisfactory result of microwave method was because the nucleus and cytoplasmic detail was unclear, and this might be due to inadequate tissue fixation in formalin and fatty tissue dropout whereas in our study, the unsatisfactory histopathology slides are mainly due to tissue degeneration of the samples.[6]

The percentage difference between microwave oven method and conventional method of tissue processing in our study and researcher Harsh Kumar study is also similar in a way as both project as a percentage difference of around 4% between the two methods.[9] All the studies mentioned above have used human samples and their results are comparable to our study.

The current study is limited by the usage of animal tissue and the number of samples processed. A larger number of samples and human samples of various types and sizes will provide more insight into the usage of the microwave-assisted tissue processing.

CONCLUSION

We concluded that we concluded that Group 2 microwave protocol is the most appropriate protocol (Protocol A) for tissue processing procedure when using domestic microwave oven (Sharp microwave oven, model no: R270EK). This is because the overall percentage of satisfactory slides inmicrowave protocol group 2 is significantly higher than microwave protocol groups 3 and 4. The morphological

quality of histopathology slides is best observed when the tissue processing time for microwave tissue processing is 62 minutes as compared to 70 minutes and 77 minutes. The quick tissue processing time for microwave tissue processing protocol increases efficiency and reduces both the cost of reagent use and patient anxiety. Histopathology slides of group 2 microwave protocol are closely comparable to histopathology slides of group 1 conventional protocol.

ETHICS APPROVAL

The study was approved by International Medical University Joint committee for ethics.

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International Medical University Malaysia Authors' contribution: All authors had access to the data and an important role in writing the paper. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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