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Original Article

Pathology Section

Domestic Microwave Versus Conventional Tissue Processing: A Quantitative and Qualitative Analysis

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ABSTRACT

Background: Microwave irradiation has been tried as a replacement for the conventional tissue processing technique in histopathology laboratories for quite some time. Studies have shown that Domestic Microwave Tissue Processing (DMWTP) provides a faster delivery of the tissue sections with a morphology which is similar to that which is seen Conventional Tissue Processing (CTP). But many laboratories still confine the domestic microwave tissue processing method only to the handle selected specimens, for which urgent reports are needed. One of the probable reasons is that, understanding about the number of tissue sections which can be processed using a microwave oven at a time, with the appropriate quality, still remains unclear.

Aim: The aim of this study was to quantitatively analyze the optimum number of samples that a domestic microwave could process at a time, as well as to qualitatively analyze the morphological outcome of those tissue sections with that of conventional processing.

Materials and Methods: This study was approved by the research and ethical committee of Sree Balaji Medical College and Hospital. A total of 135 paired tissue sections were included in the study. Ten tissue sections (which are mentioned hereafter as A10) were processed in a domestic microwave and their paired 10 tissues were processed by a conventional method. Subsequently, the number of tissues which was to be processed was increased to B15, C20, D25, E30 and F35, after ascertaining that the morphological qualities of the previously processed tissue sections were satisfactory. Sections of 4 µm thickness were taken and they were stained by the Haematoxylin and Eosin method. The slides of the tissues which were processed by the microwave method and the conventional method were randomly numbered, for a blind study, which were independently evaluated by two observers. The qualities of slides were assessed, based on 4 parameters: the cytoplasmic details, the nuclear details, the tissue architecture and the staining characteristics. The statistical analysis was done by using SPSS 15.0.

Results: The morphological outcomes (quality) of the DMWTPs were comparable to that of the CTPs, when the sample load (quantity) in the microwave oven was up to 25 samples.

Conclusion: Domestic microwave processing can be effectively used in laboratories with a maximum sample size of 25 samples per load. This has the advantage of being rapid, with its morphological quality being identical to that of conventional processing.

Key Words: Domestic microwave oven, Tissue processing

INTRODUCTION

Microwaves are becoming an integral part of our lives. A microwave (MW) is a form of nonionizing radiation that produces alternating electromagnetic fields that result in the generation of instantaneous heat, thereby helping in the faster cooking of biological materials.

The histological fixation of tissues by using microwave energy started as early as 1970 [1]. Since then, microwaves have been tried as alternatives to the conventional tissue processing techniques [2,3].

Studies have shown that MW tissue processing is a means for a faster delivery of tissue sections, with the quality of the microscopic tissues being identical to that of those sections which are processed by conventional methods [4-6]. Fully automated microwave assisted tissue processors have come into vogue, to cater to laboratories with higher sample throughputs [7-9], but still, the medium sized laboratories rely largely on conventional tissue processing and they use microwaves only for selected cases that need urgent reporting. This resistance could be due to the uncertainty that prevails about the number of tissues that can be processed by a DMW at a time, with good microscopic quality.

The present study was undertaken in an attempt to fill this lacuna, by analyzing the optimum number of samples that could be processed by DMWs at a time, with an effective morphological outcome.

OBJECTIVES

1. To find whether the DMW method would be reliable in place of a conventional method for histoprocessing.

2. To determine the optimum number of tissues that could be processed by DMWs, with statistically significant morphological outcomes in comparison to those of conventional methods.

MATERIALS AND METHODS

Study place: The Histopathology Laboratory, Department of Pathology, in a tertiary hospital, Chennai, India.

Study period: July 2012 – August 2012.

Study samples

Inclusion criteria

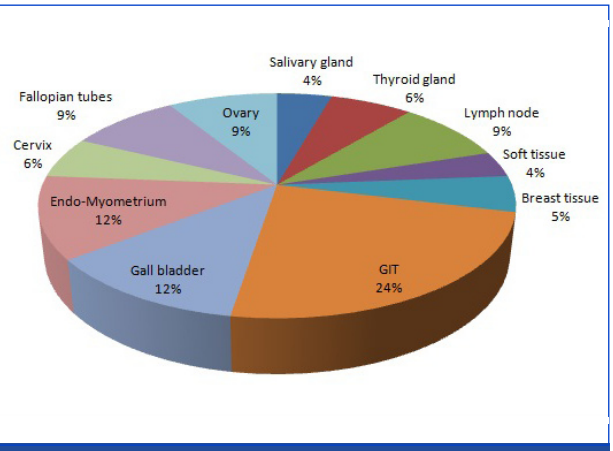
Soft tissues specimens and tissues which were more than 2 cm size were randomly selected and included in the present study.

Exclusion criteria

Small biopsy specimens (TURP chips, endometrial curettings, cervical biopsies, oral and skin biopsies) were excluded.

The tissue sections from different organs were included, as shown in [Table/Fig-1]. Tissue bits with a size of 1* 1* 0.5 cm were taken [10], which included neoplastic and non-neoplastic lesions. Those tissues which were processed by microwaves and their paired tissues which were processed by conventional methods were grouped into groups I and II respectively. The utmost precautionary measures were exercised in the laboratory, especially while chemicals were handled and during the microwave processing.

[Table/Fig-2] shows the steps of the histoprocessing technique (DMWTP and CTP) which were followed. The time which was taken for the processing by both the methods has been represented in [Table/Fig-3].



[Table/Fig-1]: Details of tissue sections GIT- Gastro Intestinal Tract

Processing Steps	Conventional Processing	DMWTP
Fixation	Formalin	Formalin
Dehydration	Iso propyl alcohol 70% 80% 90% 100%	100% Isopropyl alcohol + acetone
Clearing	Xylene I Xylene II	Xylene
Wax impregnation	Paraffin I Paraffin II	Paraffin

[Table/Fig-2]: Comparison of processing steps between DMWTP and CTP

The Conventional Tissue Processing technique (Manual method)

The specimens were left in 10% neutral buffered formalin for overnight fixation, followed by dehydration in graded isopropyl alcohol (70%, 80%, 90%, 100%), clearing with two changes of xylene and finally, impregnation with two changes in paraffin. All the processing was done at room temperature, except for the impregnation and the embedding, which were done at 56°C [11].

The Domestic Microwave Tissue Processing technique

A routine domestic microwave oven, which is used for cooking purposes, was used for the histopathology processing in our study. A microwave oven: LG microwave appliance, Model no.: MS – 2029uw (ADRQEIL); Serial no.: 112EMYA004394; Input – 1200W, 5.2A, 230V, 50 Hz; Output – 800W, 2450 MHz; Mfd by LG Electronics India Pvt Ltd; was used for this study. Tissues in plastic cassettes were placed in the microwave resistant (one litre) container at equi-distance from one another, as overlapping could hinder the diffusion of the solution into the tissues. The samples were microwaved for a few minutes in 10% neutral buffered formalin [Table/Fig-3], despite a prior fixation with formalin for 12 hours at room temperature. This was done to ensure adequate tissue fixation before the processing was started. Then, the tissues were microwaved with a mixture which contained 100% isopropyl alcohol and acetone (equal quantities) for dehydration. The clearing was done with a single change of xylene, followed by wax impregnation.

Initially, the procedure was standardized for the baseline timing, by doing a pilot study with 10 samples; The baseline timings were fixed at 2, 40, 10 and 30 minutes, for fixation, dehydration, clearing and impregnation respectively.

A temperature of 50°C - 65°C was programmed at a pressure of 75kPa and after each step, the microwave was allowed to cool for 1 minute, before proceeding to the next step of the processing. This was done to avoid overheating of the solutions. Fresh solutions were used at each step of the processing, in this study. Separate containers were used at each step of the processing. The volumes of the reagents were made up in such a way that all the tissue cassettes were completely immersed in them. After each step, the changes in the colour and the consistency of the tissue were observed. Sections of 4-5µm thickness were taken from the paraffin embedded tissue blocks that were processed by both the techniques by using a Leica rotary microtome. They were stained with Haematoxylin and Eosin.

METHODOLOGY

The method of evaluation of the processed slides

For objective 1: The slides were randomly numbered for a blind

Processing Steps	Conventional Method (Hours) I	Microwave Processing (Minutes) II					
		A	B	C	D	E	F
Fixation*	-	02	04	06	08	10	12
Dehydration	1+1+1+1=4 hours	40	45	50	55	60	65
Clearing	1+1=2 hours	10	15	20	25	30	35
Wax impregnation	30 min + 30 min= 1 hours	30	30	30	30	30	30
Total processing time	7 hours	1 hour 22 min	1 hour 34 min	1 hour 46 min	1 hour 58 min	2 hours 10 min	2 hours 22 min

[Table/Fig-3]: Duration of processing by conventional method and microwave method * - Prior fixation for ≥12 hours with 10% formalin was done for both CTP and DMWTP;

Parameters	Features
Tissue architecture	Stroma; Inflammatory cells; Red cell lysis; Secretory products
Cytoplasm	Eosinophilia of cytoplasm; Nuclear – cytoplasmic contrast
Nucleus	Chromatin condensation; Nuclear membrane; Mitotic figures
Staining characteristics	Eosinophilic cytoplasm, Crisp staining of nucleus, Nuclear cytoplasmic contrast

[Table/Fig-4]: Details of morphological parameters with its features

Analysis (N = 135)	Test (P-value)
Quantitative: Reliability Analysis DMWTP CTP	Cronbach's α 0.995 (0.000)* 0.975 (0.000)*
Qualitative: Measure of Agreement DMWTP CTP	Kappa Statistics 0.984 (0.000)* 0.934 (0.000)*

[Table/Fig-5]: Reliability Analysis and Measurement of Agreement between the evaluators

study. Two experienced pathologists evaluated all the slides without having any knowledge on the processing techniques which were used. The qualities of the slides were assessed, based on 4 morphological parameters: the cytoplasmic details, the nuclear details, the tissue architecture and the staining characteristics, as has been represented in [Table/Fig-4].

The scores which were assigned by the evaluators after the microscopic examination of the slides were as follows:

Score 2 (Good) – if three of four parameters were satisfied

Score 1 (Fair) – if one or two parameters were satisfactory

Score 0 (Poor) – if none of the parameters were satisfactory

For objective 2: To find the optimum load of tissues in a DMWTP which had a similar morphological quality as that which was seen in the conventional technique, the number of paired tissues for the processing was increased gradually from a sample size of 10, 15, 20, 25, 30 and 35 simultaneously; they were grouped into groups I (for DMWTP) and II (for CTP) of A(10), B(15), C(20), D(25), E(30) and F(35) respectively. The duration of the processing with each sample size was gradually increased [Table/Fig-3]. Only when the histomorphology was satisfactory, the sample size was further increased.

STATISTICAL ANALYSIS

The observed data was analyzed by SPSS 15.0 and the scores which were obtained for both the techniques were presented with descriptives (as frequency, mean, standard error) [12], with inferential tests (like Reliability Analysis – Cronbach's α [13] ; Measure of Agreement – Kappa statistics) for the interobserver variation and with the Student's-t-test (the quality of the DMWTP and the CTP sections) for the independent samples. A type I error α of 5% was considered as the level of significance.

RESULTS

The number of tissue bits which were taken from various organs has been enumerated in [Table/Fig-1]. GIT samples constituted the most (around 36%) numbers of tissue bits, whereas soft tissue samples were the least in number (4%).

For Objective 1: The interobserver variations in assessing the histomorphological quality of the tissues which were processed by DMWTP and CTP were tested quantitatively by 'Reliability analysis', where Cronbach's α was 0.995 (P=0.000) for DMWTP and Cronbach's α was 0.975 (P=0.000) for CTP. There existed a statistically significant 'Measure of Agreement' between the evaluators, when they were tested qualitatively with a Kappa value of 0.984 (P=0.000) for DMWTP and a Kappa value of 0.934 (P=0.000) for CTP, as has been presented in [Table/Fig-5].

For Objective 2: We averaged the scores of the two evaluators which were provided for both the techniques (DMWTP and CTP) separately, for all the six groups (A10 to F35). [Table/Fig-6] shows mean±SE (mean) scores which were obtained for the two techniques for the groups of different sample sizes. We observed a value of 1.8±0.13 for DMWTP and a value of 1.6±0.22 for CTP, which yielded a statistically insignificant difference with t=0.77(P=0.449) for the paired samples of 10 tissues. When we increased the sample size from 15 to 25 tissues for groups B, C and D, no difference was observed in the mean score, with a slight variation in the SE for both the techniques. When the paired tissues of 30 and 35 were processed, we obtained a statistically significant difference in the mean scores of the histological quality between the DMWTP and the CTP with t=2.87 (P=0.006) and t=5.50 (P=0.000).

DISCUSSION

In the present study, a total of 270 tissue bits were processed totally [Table/Fig-1].

GIT and FGT (Female genital tract) specimens constituted the main bulk of the tissues, together making seventy two percent of the total samples. This simulates the routine sample load in a histopathology laboratory. Smaller biopsies were excluded from the study, as they could interfere with the optimization of the time schedule for the microwave processing (because small biopsies will be processed earlier than the large tissue bits, and so, when they are allowed to be processed till the large bits are processed, they are likely to be charred.) Moreover, taking paired tissue bits also pose difficulties.

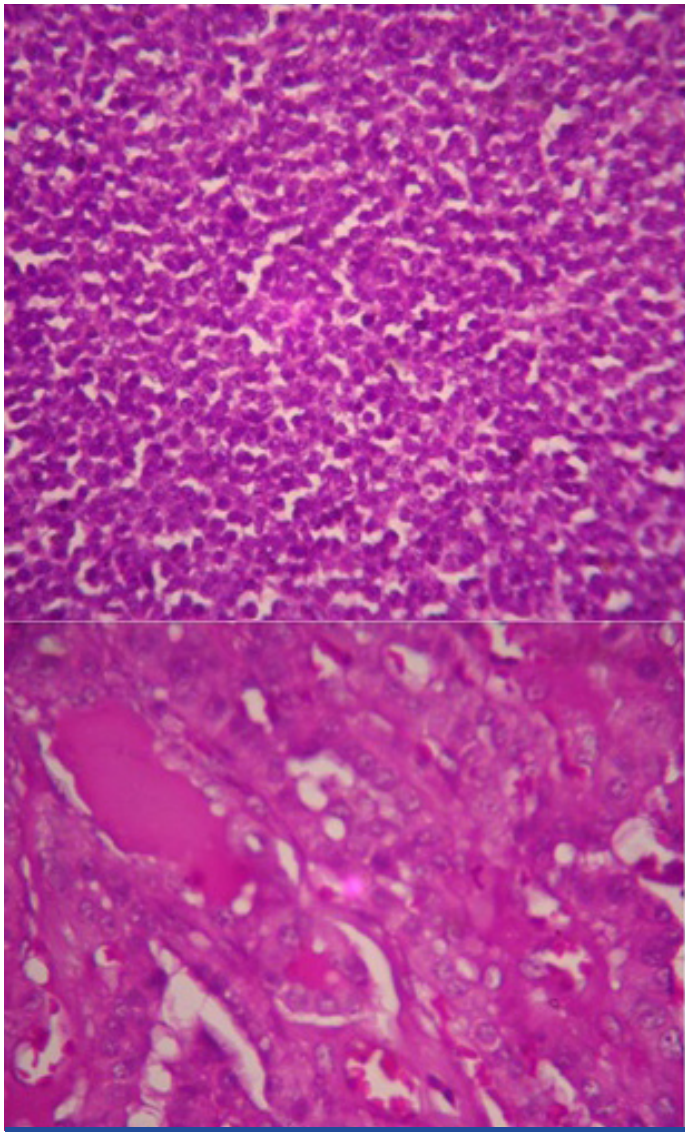
The CTP was done by using a standard protocol [11]. For the microwave histoprocessing, various authors have used different protocols [5]; In the present study, isopropyl alcohol and acetone were used for dehydration, xylene was used for clearing and paraffin was used for embedding [Table/Fig-2]. A mixture of equal amounts of acetone and isopropyl alcohol was used in the DMWTP, in order to enhance the quality of the dehydration. Though few authors had utilized chloroform for clearing [5], in the present study, the clearing was done with xylene, with satisfactory results. It was observed that a longer time was needed for the wax impregnation (30 minutes) as compared to that in the previous studies, where it ranged from five minutes to fifteen minutes [14].

The total time which was taken for the microwave processing, increased gradually with an increase in the number of samples, from about 1 hour and 22 minutes to 2 hours and 22 minutes. Still, the turnaround time was observed to be very less as compared to the conventional processing, which took nearly seven hours [Table/Fig-3].

Plastic cassettes were exclusively used for the microwave processing, as metallic cassettes could cause a risk of sparking and even explosion.

No. of Sample	Processing method	Observer 1			Observer 2			Mean±SE(Mean)	t-value (P-value)
		Scores			Scores				
		0	1	2	0	1	2		
A (10)	DMWTP (I)	-	2	8	1	2	8	1.8±0.13	0.77 (0.449)
	CTP (II)	1	2	7	1	2	7	1.6±0.22	
B (15)	DMWTP (I)	-	3	12	-	2	13	1.8±0.09	0.23 (0.816)
	CTP (II)	-	3	12	-	3	12	1.8±0.10	
C (20)	DMWTP (I)	-	3	17	-	3	17	1.8±0.08	0.67 (0.503)
	CTP (II)	1	3	16	1	3	16	1.7±0.12	
D (25)	DMWTP (I)	-	3	22	-	3	22	1.8±0.06	0.22 (0.821)
	CTP (II)	-	2	23	-	3	22	1.9±0.05	
E (30)	DMWTP (I)	3	8	19	3	8	19	1.5±0.12	2.87 (0.006)*
	CTP (II)	-	2	28	-	3	27	1.9±0.04	
F (35)	DMWTP (I)	6	15	14	6	15	14	1.2±0.12	5.50 (0.000)*
		-	2	33	-	2	33	1.9±0.03	

[Table/Fig-6]: shows no. of samples scored 0, 1 and 2, processed by DMWTP and CTP and Student T test for different sample sizes *Significant



[Table/Fig-7]: shows the microscopic picture of sections processed using DMW (40X)

A- Non Hodgkin Lymphoma of Tonsil (B-15) showing distinct morphology and satisfactory staining features (score 2)

B- Papillary carcinoma thyroid (E-30) showing less satisfactory cellular morphological and staining features (Score 1)

It was observed that the shrinkage of the tissue bits was more pronounced with the DMWTP as compared to the CTP, but this did not affect the morphological quality of the tissues. This was consistent with the observations of other authors [10].

Based on the microscopic parameters which were enumerated, in [Table/Fig-4], scores which ranged from 0 to 2 were assigned to each of the tissue sections by two independent evaluators. The 'Reliability Analysis' and the 'Measurement of Agreement' between the evaluators were assessed by using Cronbach's α and Kappa values respectively [Table/Fig-5]. Both were found to be statistically significant (P=0.000). This showed that the morphological outcome of the DMWTP was very much comparable to that of the CTP qualitatively (Objective 1).

[Table/Fig-6] shows the comparison of the two methods, with the microscopic scores and the mean ±SE values for different sample sizes. No difference was observed in the mean score, with a slight variation in the SE for both the techniques, when up to 25 samples were run as a load. When the paired tissues of 30 and 35 were processed subsequently, we obtained a statistically significant difference in the mean scores of the histomorphological quality between the DMWTP and the CTP with t=2.87 (P=0.006) and t=5.50 (P=0.000) respectively. This validated our second objective that the DMWTP technique produced a similar histomorphological quality as that of the CTP technique, for an optimum sample size of twenty five samples. Furthermore, it was observed that the mean score remained consistent at 1.8 with the DMWTP for the sample sizes A (10) to D (25), whereas that the mean for the CTP varied from 1.6 to 1.9. This showed clearly that the morphologies of the DMW processed tissue sections were consistently good when the sample load was up to 25. Two microscopic pictures of the sections which were processed with the DMWTP have been included below [Table/Fig-7] shows the difference in the morphology with sample sizes of IB(15) and IE(30).

CONCLUSIONS

In comparison to the CTP, the DMWTP method is more reliable in terms of the tissue architecture, the nuclear details, the cytoplasmic details and the staining quality, when the sample size is up to 25 per load. The turnaround time is drastically reduced with the DMWTP as compared to that of the CTP. However, when the

sample load is increased further in the DMW, the quality of the tissue sections decreases.

Limitations and Future Recommendations

Greater caution has to be exercised while the microwave is handled, to avoid accidents. Small biopsy samples were not included in the present study. Doing a study which involves the microwave processing of such tissue samples, along with that of regular tissues, may throw more light in this area.

ABBREVIATIONS

Domestic Microwave=DMW
Tissue Processing= TP
Conventional Tissue Processing = CTP

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