Original Article

Rapid Microwave Tissue Processing and Staining Method Using a Kitchen Microwave Oven in Histopathology Laboratory: A Comparative Study with Routine Histoprocessing Method

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Abstract

Background: Histopathological diagnosis of a specimen requires good histoprocessing. It ultimately depends on sample preparation and staining, which is time consuming by routine tissue processing technique. The objective of the present study was to compare the reliability of kitchen microwave for tissue processing and staining with the standard conventional processing and staining in different tissue samples. **Materials and Methods:** The study sample included 204 specimens from different parts of the body. Three set of tissues were processed and three set of slides from each sample were stained by routine as well as microwave method and the quality of microscopic features was compared. **Results:** No significant difference was found in nuclear and cellular characters in case of staining by both methods. While, in case of processing, mean scoring for nuclear characters for routinely processed and microwave-stained (RM) slides was 6.95 ± 0.09 (confidence interval [CI] 6.83–0.09), for microwave-processed and microwave-stained (MM) slides, it was 6.82 ± 0.07 (CI 6.74–6.90) by observer 1 (P = 0.068). The mean score of cellular character of RM slides was 7.85 ± 0.06 (CI 7.76) and for MM slides, it was 7.65 ± 0.17 (CI 7.47–7.88) by observer 2 (P = 0.076). The microwave method was better for both processing (P = 0.001) and staining (P = 0.003) in case of small biopsies (P = 0.057). The microwave method was as good as routine method for processing of aorta and staining of breast and kidney tissues (P = 0.23, 0.32, and 0.38, respectively). The time taken by the microwave method is considerably less for both processing and staining. **Conclusion:** The microwave method requires less time when compared with the routine method, thus reducing the overall turnaround time which ultimately helps in rapid same-day diagnosis and patient management. Microwave can be preferred method for smaller biopsies when compared with large biopsies. Lack of automation and requirement of more manpower remain the major disadvantages for use of microwave method in large turnover histopathology laboratories.

Keywords: Histoprocessing, kitchen microwave, processing, staining

Introduction

The journey of tissue processing and staining in histopathology was started in the 19th century. The journey started with the conventional tissue processing, to frozen sections, to the automated tissue processing, and now to the microwave application in the field of histotechniques for fixation, processing, and staining of surgical samples.[1,2] As the routine manual histoprocessing is time-consuming, an alternative method like microwave tissue processing and staining can play a vital role in the rapid diagnosis by a pathologist.[2]

The microwave works on the principle that the electromagnetic field leads to the excitation of molecules which brings about its rotatory movement.[3] The rotation of molecule causes friction between them and produces energy in the form of heat within the

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materials. This internally generated heat, in contrast to conventional heating, enhances the rate of diffusion of fluids in and out of the tissue sections more effectively in histotechniques.[3,4]

If we can switch from the routine overnight technique for the tissue processing and staining to same-day microwave method, it can substantially reduce the turnaround time which ultimately will lead to same-day diagnosis. This will help in rapid patient diagnosis and management.[5] The kitchen microwave oven improvement will reduce not only the turnaround time, but also the costs associated with diagnosis and less chances for patient dissatisfaction, which by default could lead to hastening the initiation of therapy, especially in neoplastic diseases.

On literature review, there are many studies which have analyzed the microwave processing of tissue[3-6] and only few studies which have evaluated the microwave staining technique.[6,7] In all of these studies, the sample size was small. In this regard, we could not find any study with large sample size from different organs. Thus, the present study intends to compare the quality of routine tissue processing and staining with rapid microwave method using a kitchen microwave oven in different tissues at a tertiary care center in Central India.

Figure 1: Microwave used in processing in the study

Materials and Methods

Study design

The present study was a laboratory-based observational study conducted in the histopathology section of Department of Pathology at Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha, Maharashtra, a rural tertiary care hospital in Central India over the period of 18 months after clearance from the Ethics Committee of the Institute.

One or two random specimens per day (every 2nd and/or 8th specimen) were selected for the present study following inclusion and exclusion criteria. The extracted pair of tissue section was processed routinely[8] in an automated tissue processor (Myr-STP 120—Histokinete) as well as by the microwave method. The kitchen microwave oven used was BPL-SANYO, model number OVEN-01 [Figure 1], and the power used in each step was to the lowest (100 W). The tissue block was obtained by paraffin wax embedding and was sectioned accordingly by microtome. Ribbons of the section were taken on two set of slides using hot water bath; the sections were de-waxed. For tissue processing, we used a glass jar containing processing reagent with tissues wrapped in a filter paper and dipped in the reagent, whereas for microwave staining, we used a coplin jar. The first set of the slides was processed routinely and stained with Hematoxylin and Eosin (H&E) in a routine manner[7] (Protocol I) [Table 1]. The first set of slides was from routinely processed and routinely stained slides (RR). The second set of slides was routinely processed but were stained with H&E using microwave schedule[7] (Protocol II) [Table 1]. It was termed as routinely processed and microwave-stained slides (RM). The third set of slides was both processed by and stained with H&E using the microwave method[8] [Table 2]. It was termed as microwave-processed and microwave-stained (MM) [Figures 2–6].

Inclusion criteria

1. All random specimens were received for histopathology diagnosis where the tissue was adequate for the study after setting aside for normal (routine) processing,

Table 1: Staining by routine and microwave method[7]

Step no.	Reagents	(Protocol I) Routine staining technique	(Protocol II) Microwave staining technique
1	100% IPA	15 min	3 min
2	Water	10 min	3 min
3	Hematoxylin	10 min	1 min
4	Blueing	10 min	1 min
5	Acid alcohol	1 dip	1 dip
6	Water bath	10 min	1 min
7	Eosin	1 dip	1 dip
8	Alcohol	1 dip	1 dip
9	Xylene	10 min	10 min (not in microwave, as it is combustible)
Total time taken		Approximately 1 h 5 min	Approximately 20 min

Table 2: Microwave method: Processing schedule[8]

Step no.	Reagent used	Duration (min)
1	100% reagent grade ethanol/alcohol at 360 W	10
2	100% reagent grade ethanol/alcohol at 360 W	10
3	100% isopropyl alcohol at 360 W	10
4	100% isopropyl alcohol at 360 W	10
5	Molten paraffin wax at 180 W	10
6	Molten paraffin wax at 180 W	10
Total time taken		60

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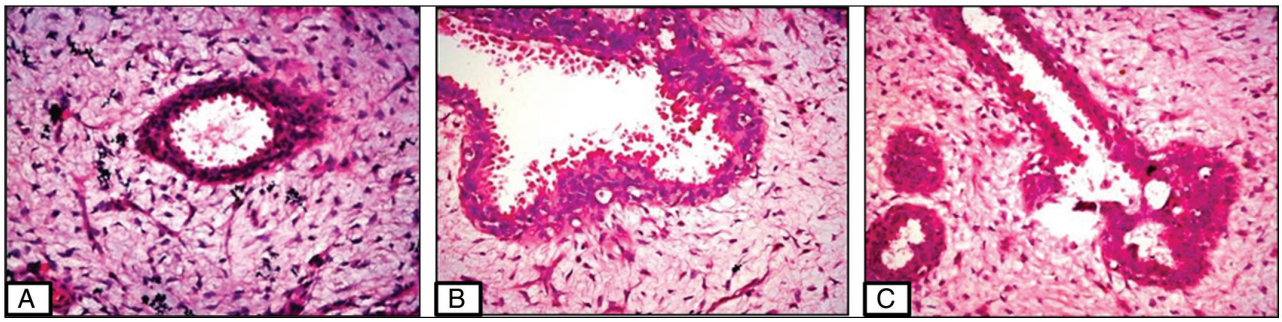

Figure 2: Histoprocessing of small biopsy from breast tissue along with pathologist 1 and 2 scoring, respectively. (A) Routinely stained routinely processed (score: 6.71/7.52) (H&E, 400X), (B) routinely stained microwave processed (score: 6.71/7.52) (H&E, 1000X), and (C) microwave stained routinely processed (score: 7.27/8.14) (H&E, 400X)

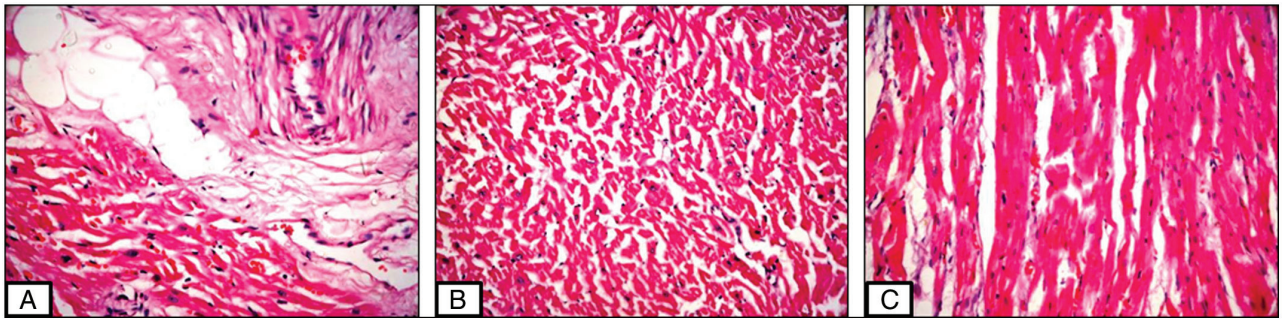

Figure 3: Histoprocessing of large biopsy from heart tissue along with pathologist 1 and 2 scoring, respectively. (A) Routinely stained routinely processed (score: 6.86/7.81) (H&E, 400X), (B) routinely stained microwave processed (score: 6.96/7.85) (H&E, 100X), and (C) microwave stained routinely processed (score: 7.17/8.18) (H&E, 400X)

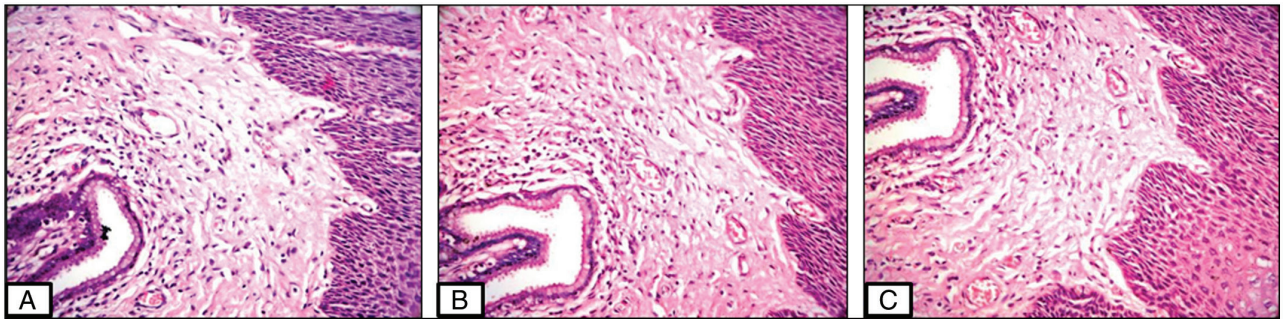

Figure 4: Histoprocessing of small biopsy from cervix along with pathologist 1 and 2 scoring, respectively. (a) Routinely stained routinely processed (score: 6.32/6.98), (b) routinely stained microwave-processed (score: 6.91/7.77), (c) microwave-stained routinely processed (score: 6.94/8.08) (H&E, 400×)

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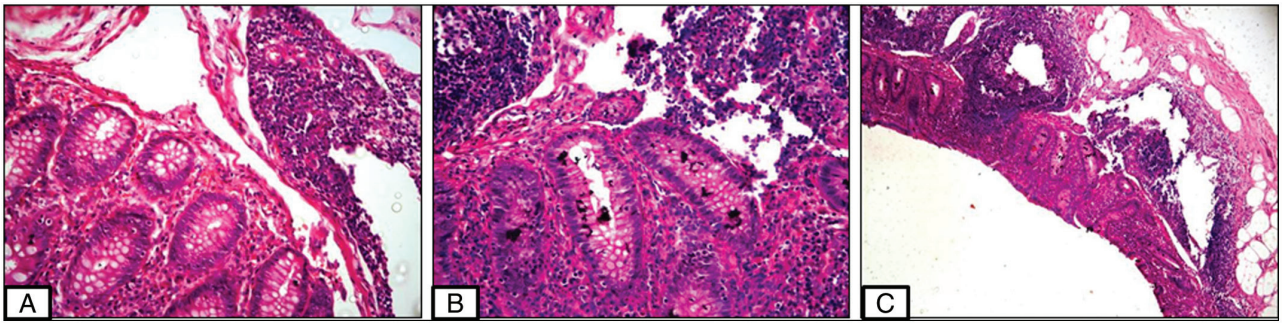

Figure 5: Histoprocessing of large biopsy from appendix along with pathologist 1 and 2 scoring, respectively. (a) Routinely stained routinely processed (score: 7.37/8.37) (H&E, 400×), (b) routinely stained microwave-processed (score: 7.12/8.12) (H&E, 400×), (c) microwave-stained routinely processed (score: 7.33/8.58) (H&E, 100×)

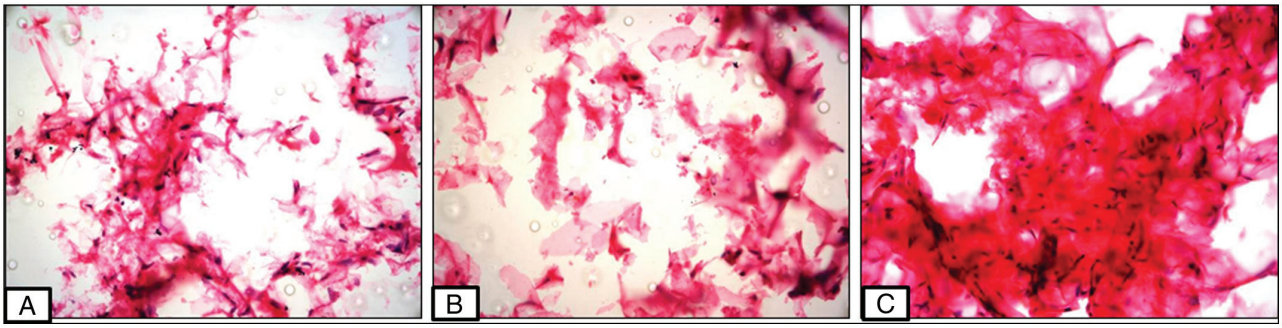

Figure 6: Histoprocessing of small biopsy from fatty tissue along with pathologist 1 and 2 scoring, respectively. (a) Routinely stained routinely processed (score: 6.56/7.43) (H&E, 400×), (b) routinely stained microwave-processed (score: 7.12/8.12) (H&E, 400×), (c) microwave-stained routinely processed (score: 8.25/9.12) (H&E, 400×)

staining and diagnosis to make two extra sections of size ≥10 × 5×2 mm.

2. All cases in which the random specimen was selected but the tissue was not adequate enough for taking two extra sections for the study; in these cases, the representative tissue was taken from the excisional specimens in case of cervical biopsy or endometrial biopsy from the hysterectomy specimens, in case of skin biopsy from amputations or modified radical mastectomy specimens, in case of liver or kidney biopsy from autopsy liver and kidney specimens.
3. Cases in which tissues were sent for frozen section, if they were adequate enough to make two extra sections of size 10 × 5 × 2 mm.

Exclusion criteria

Biopsy specimens in which three sets of slides could not be prepared due to any reason were excluded from the study.

Out of the 221 samples received, 3 set of slides could not be obtained in 17 samples, so these were excluded from the study. Remaining 204 samples were included in our present study following strict inclusion and exclusion criteria.

All the slides were coded (only researcher knew it) and the pathologists who did the scoring were kept completely blinded regarding which method of staining and processing used. Each pathologist evaluated the tissue section for cellular details such as size of cells, cellular outline, clarity, cytoplasmic details, and nuclear details such as size of nucleus, clarity of nucleus, and clarity of nuclear membrane.[7] All parameters were scored on a scale from minimum 1 to maximum 10 (1–4.5= poor, >4.5–6= suboptimal, ≥6–9= good, and >9–10= excellent).[7]

Statistical analysis

The statistical analysis was performed by using descriptive (such as frequency, mean, standard deviation) and inferential statistics using Student's unpaired *t*-test for the independent samples and reliability analysis—Cronbach's alpha and software used in the analysis was SPSS 22.0 (IBM Corp., Released 2015, IBM Statistics for Windows, Version 20.0, Armonk, NY, USA) and GraphPad PRISM 5.0 version, and $P < 0.05$ is considered as the level of significance.

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form

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were reviewed and approved by a Institutional Ethics Committee according to the document number MGIMS/ IEC/ PATH/101/2016, dated 05/10/2016 to get this approval. The sections used in this study were not used for diagnosis, and the study was done in such a way that would not hamper the routine reporting and diagnosis. Patient confidentiality was maintained during all research procedures.

Results

Out of the 204 samples, cervical biopsies (n = 34, 16.67%) constituted the maximum among the study samples. The other organs included aorta (n = 21, 10.29%), endometrial biopsies and breast (n = 20, 9.80% each), liver (n = 15, 7.35%), lung (n = 13, 6.37%), kidney (n = 12, 5.88%), heart and spleen (n = 10, 4.9% each), colon and salivary gland (n = 8, 3.92% each), ovary and uterus (n = 6, 2.94% each), lipoma (n = 4, 1.96%), gall bladder and appendix (n = 3, 1.47% each), brain, large intestine, skin, and adrenal gland (n = 1, 0.49% each), and other (n = 7, 3.43%).

For comparison of processing, RM and MM slides were compared. The mean scoring for nuclear characters for RM slides was 6.95 ± 0.09 (CI 6.83–0.09) and for MM slides, it was 6.82 ± 0.07 (CI 6.74–6.90) by observer 1 (P = 0.068). The mean score of cellular character of RM slides was 7.85 ± 0.06 (CI 7.76) and for MM slides, it was 7.65 ± 0.17 (CI 7.47–7.88) by observer 2 (P = 0.076). RM slides were significantly better than MM slides by observer 1 for nuclear characters (P = 0.068) and for cellular characters by observer 2 (P = 0.076) [Table 3, upper part].

On comparison of staining, there was no difference in nuclear as well as staining characters by both observers in RR and MM slides. The mean scoring for nuclear characters for RR slides was 6.89 ± 0.16 by observer 1 (CI 6.74–7.10) and 7.87 ± 0.20 by observer 2 (CI 7.68–8.15). The mean score of cellular characters of RR slides was 7.05 ± 0.14 by observer 1 (CI 6.88–7.24) and 7.94 ± 0.09 by observer 2 (CI 7.88–8.09). Similarly, the mean scoring for nuclear character for RM slides was 6.95 ± 0.09 by observer 1 (CI 6.83–7.06) and 7.78 ± 0.08 (CI 7.70–7.89) by observer 2. The mean score of cellular characters of RM slides was 7.08 ± 0.07 by observer 1 (CI 7–7.15) and 7.85 ± 0.06 by observer 2 (CI 7.76–7.92). The P-values for nuclear features were 0.52 and 0.47 by observers 1 and 2, respectively, whereas for cellular features, P-values were 0.66 and 0.16 by observers 1and 2, respectively [Table 3, middle part].

The inter-observer variations in assessing the histological quality of the tissues which were stained by RR, RM, and MM were tested quantitatively by "reliability analysis," in which Cronbach's α for RR was 98.3% for observer 1 and 90.4% for observer 2. Cronbach's α for RM was 74% for observer 1 and 74.1% for observer 2. Cronbach's α for MM was 71.7% for observer 1 and 71.8% for observer 2. As the α-values are matching with observer 1 and observer 2, it was concluded that the present analysis was reliable [Table 3, lower part].

Table 3: Comparison of mean score for processing and staining by routine and microwave methods by observers 1 and 2 and reliability analysis between two observers

Comparison of processing						
		RM		MM		*P-value
		Mean±SD	95% CI	Mean±SD	95% CI	
Nuclear characters	Observer 1	6.95±0.09	6.83–7.06	6.82±0.07	6.74–6.90	0.068
	Observer 2	7.78±0.08	7.70–7.89	7.71±0.13	7.55–7.84	0.39
Cellular characters	Observer 1	7.08±0.07	7–7.15	6.97±0.12	6.85–7.15	0.18
	Observer 2	7.85±0.06	7.76–7.92	7.65±0.17	7.47–7.88	0.076
Comparison of staining						
		RR		RM		P-value
		Mean±SD	95% CI	Mean±SD	95% CI	
Nuclear characters	Observer 1	6.89±0.16	6.74–7.10	6.95±0.09	6.83–7.06	0.52
	Observer 2	7.87±0.20	7.68–8.15	7.78±0.08	7.70–7.89	0.47
Cellular characters	Observer 1	7.05±0.14	6.88–7.24	7.08±0.07	7–7.15	0.66
	Observer 2	7.94±0.09	7.88–8.09	7.85±0.06	7.76–7.92	0.16

Reliability analysis between the two observers		
	Observer 1 alpha (α)	Observer 2 alpha (α)
RR	0.983	0.904
RM	0.740	0.741
MM	0.717	0.718

*P<0.05 is considered as level of significance

SD: standard deviation, CI: confidence interval, RR: slides routinely processed and routinely stained, RM: slides routinely processed and stained using microwave schedule, MM: slides processed and stained using microwave method

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In case of small biopsies, the mean scores by the microwave method were significantly higher than the routine method for both processing ($P = 0.001$) and staining ($P = 0.003$). In case of large biopsies, the MM method was better than the RM in case of processing ($P = 0.057$) and it was statistically insignificant when compared between RR and RM for staining ($P = 0.77$) [Table 4 and Figures 1–4].

The different tissues were compared for processing and staining by both methods. In case of processing, only aorta showed RM results as good as RR method ($P = 0.23$); whereas in rest of the organs (breast, liver, and kidney), the routine method was superior ($P = 0.04$, <0.0001, and 0.007, respectively) [Figure 1]. In case of staining, breast tissue and kidney tissue showed MM results as good as RM method ($P = 0.32$ and 0.38, respectively); whereas in the case of aorta and liver, the routine method was superior ($P \leq 0.0001$ each) [Table 5].

As per the protocols we followed, the total time taken for processing by the microwave method was only 60 min [Table 2], compared with 20 h 30 min (after overnight fixation) by the routine method. Similarly, in the case of staining, the microwave method takes around 20 min when compared with the routine staining, which takes around 65 min [Table 1].

Discussion

We assessed difference in microscopic features by processing and staining by both routine and microwave methods in different organs in our study. Estrada *et al.*[9] used kidney, liver, skin biopsies for the microwave staining. Cavusuglov *et al.*[10] used only kidney tissues for studying the role of microwave in H&E staining. The reason for including different tissues in the present study and also in the other previous studies[10-12] was mainly to assess the effect of microwave in these varied tissues, which are received routinely in the histopathology section.

To compare the effect of microwave in processing, we kept the staining of the tissues by microwave method as a common factor (RM vs. MM). We did not find significant difference in nuclear as well as cellular characters by the microwave method in processing [Table 3]. Our findings are consistent with the studies done by Babu *et al.*,[5] Shashidara *et al.*,[13] Mathai *et al.*,[14] Kumar *et al.*,[15] and Choji *et al.*[16] The physicochemical basis of tissue processing lies in the diffusion of reagents into the tissue to be processed. Microwaves, which are formed due to non-ionizing radiation, produce alternating electromagnetic fields that result in the rotation of polar molecules such as water and the polar side chains of proteins through 180° at the rate of 2.45 billion cycles/s. All domestic microwaves

Table 4: Comparison of mean scores for staining and processing by routine vs. microwave method for small and large biopsies

Type	Small biopsies ($n=63$)			Large biopsies ($n=29$)		
	Mean	SD	*P-value	Mean	SD	*P-value
Comparison of processing						
RM	7.90	0.40	0.001	7.98	0.22	0.057
MM	8.14	0.41		8.18	0.51	
Comparison of staining						
RR	7.67	0.59	0.003	7.94	0.72	0.77
RM	7.90	0.40		7.98	0.22	

*P<0.05 is considered as level of significance

SD: standard deviation, RR: slides routinely processed and routinely stained, RM: slides routinely processed and stained using microwave schedule, MM: slides processed and stained using microwave method

Table 5: Comparison of mean scores for staining and processing by routine vs. microwave method for specific organs

Organ	n	Comparison of processing				Comparison of staining			
		Type	Mean	SD	*P-value	Type	Mean	SD	*P-value
Aorta	21	RM	7.77	0.15	0.23	RR	8.27	0.24	<0.0001
	21	MM	7.67	0.35		RM	7.77	0.15	
Breast	20	RM	7.65	0.34	0.04	RR	7.52	0.47	0.32
	20	MM	8.14	0.64		RM	7.65	0.34	
Liver	15	RM	7.02	0.42	<0.0001	RR	8.46	0.29	<0.0001
	15	MM	6.01	0.65		RM	7.02	0.42	
Kidney	12	RM	8.07	0.29	0.007	RR	8.21	0.47	0.38
	12	MM	7.31	0.84		RM	8.07	0.29	

*P<0.05 is considered as level of significance

SD: standard deviation, RR: slides routinely processed and routinely stained, RM: slides routinely processed and stained using microwave schedule, MM: slides processed and stained using microwave method

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operate at 2.45 GHz, corresponding to a wavelength in vacuum of 12.2 cm.[17] The molecular kinetics so induced result in the generation of instantaneous heat that is proportional to the energy flux and continues until the radiation ceases.[18] Microwave excitation of molecules is a process in which applied energy penetrates into the tissues to a greater depth when compared with the other manual methods of tissue processing.

To compare the staining characters, we kept the processing of the tissues by the routine method as a common factor (RR vs. RM). In our study, both observers found no significant difference in the mean score between routine- and microwave-stained method for both the nuclear (P = 0.52 and 0.47, respectively) and cellular features (P = 0.66 and 0.16, respectively) [Table 3]. So, we concluded that there is no significant difference in the assessment of quality of staining by both methods. Our findings are consistent with the findings of Kango and Deshmukh[19] (P = 0.861) and Temel *et al.*[20] Kango and Deshmukh[19] found that cellular details were visualized in all the slides by observers, and intercellular bridges, brush borders, and cilia were also observed in detail in both the staining methods. Mukunda *et al.*[7] found better cellular and nuclear details by the microwave method (P = 0.031 and P = 0.001, respectively). The main reason for this discordancy may be related to the smaller sample size (20 cases), whereas in the present study, the sample size was 204 cases. Estrada *et al.*[9] studied the effect of microwave irradiation while staining the sections of various tissues such as kidney, liver, and skin biopsies by toluidine blue. They found that the microwave method had more contrast, less artifact in the form of precipitate and more uniform overall staining. These findings were consistent with the findings of our study for H&E staining.

We found statistically significant difference in processing and staining with the use of microwave (P = 0.001 and 0.003 respectively) in case of small biopsies [Table 4]. Our findings are consistent with the findings of Mukunda *et al.*[7] and Estrada *et al.*[9] This indicates that the processing and staining by the microwave method should be preferred when compared with routine histoprocessing method for small biopsies. There was no significant correlation in processing as well as in staining in case of large biopsies (P = 0.057 and 0.77, respectively) [Table 4]. Our findings are consistent with Rohr *et al.*[4] and Leong and Duncis.[21] The probable reason, for small biopsy's microwave-stained sections having increased mean score than the routine-stained sections, is mainly due to diffusion. The formula which governs the rate of diffusion is $<x2> = 2Dt$, where "x" stands for net distance covered by a particle in solution for a certain direction; t is the time period during which diffusion occurs; and D is the diffusion constant for the substance. $<x2>$ stands for the average value; so the smaller the biopsy (thickness <2 mm) better is the diffusion.[22] This may be the reason for microwave-stained sections having better mean scores.

On comparison between mean scores for assessing the processing and staining characters in individual organs by both methods, the P-value was insignificant in case of processing of aorta (P = 0.23) and staining of breast and kidney tissue (P = 0.32 and 0.38, respectively). It was highly significant for processing of breast, liver, and kidney tissues (0.04, <0.0001, and 0.007, respectively) and staining of aorta and liver tissues (<0.0001, respectively). It shows that there was no difference in processing by microwave and routine method in case of aorta and staining of breast and kidney. Kango and Deshmukh[19] and Login and Dvorak[23] also found similar results in the case of liver tissue. This may be mainly because both studies have not specifically mentioned the mean score for the liver tissues alone. The mean score mentioned was calculated as overall score for all varieties of tissues studied. Shrestha *et al.*[24] found similar results in case of breast tissue, and Kango and Deshmukh[19] (P > 0.05) and Danielson *et al.*[25] found in case of kidney tissue. We did not find any study which had studied the difference in staining for aorta.

In our study, the microwave method took around 60 min for processing when compared with the routine processing which required 20 h 65 min (after overnight fixation) [Table 2]. In case of staining, 20 min was required for microwave method, whereas 65 min was required for the routine staining method [Table 1]. Shruthi *et al.*[26] found reduction of up to 70% time by using the microwave method. Our findings are also consistent with findings of Rao *et al.*,[6] Mukunda *et al.*,[7] Panja *et al.*,[22] Shruthi *et al.*[26] and Katoh.[27] Thus, the use of microwave method leads to better turnaround time, leading to same-day reporting. It ultimately helps in rapid diagnosis, especially in the case of neoplasm leading to faster decision for the surgeon and also leading to patient satisfaction.

One of the major disadvantages of microwave method as of now is that there is no automation, so it cannot be used in laboratories with large histopathology workload and is labor-intensive as one technical staff is continuously required for this technique.

Conclusion

The microwave method requires less time when compared with the routine method, thus reducing the overall turnaround time which ultimately helps in rapid same-day diagnosis and patient management. Nuclear and cellular characteristics are better in case of routine processing when compared with microwave processing, while there is no difference in staining by both methods. Microwave staining can be a preferred method for smaller biopsies when compared with large biopsies. Lack of automation

due to which large number of samples cannot be processed at a given time and requirement of more manpower remain the major disadvantages for the use of microwave method in large turnover histopathology laboratories.

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Conflicts of interest

There are no conflicts of interest.

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