ORIGINAL RESEARCH

A Comparative Study on Microwave Tissue Processing and Conventional Tissue Processing.

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Abstract

Introduction: The field of medical technology is ever evolving which necessitates traditional techniques replaced by newer technologies. Contrary to this histotechniques in histopathology has remained static with hardly any changes where tissue preparation for microscopic examination still remains time consuming. However recent emergence of automatic tissue processor and microwaves has successfully reduced the time from several days to 1-2 days.

Materials and Methods: 133 different tissue blocks from the department of Oral and Maxillofacial Pathology were used in the current study. Each tissue received was fixed in 10% formalin overnight, sectioned into approximately two halves. One tissue was sent for routine processing whereas the other was sent for microwave processing. After processing the sections were embedded, section and stained with H and E. A pathologist evaluated the stained slides and the results so obtained were analyzed statistically.

Results: Microwave processing considerably cut down the processing time from days to merely hours. Microwave stained slides showed no loss of cellular and nuclear details, uniform-staining characteristics and was of excellent quality.

Conclusion: The cellular details, nuclear details and staining characteristics of microwave stained sections were better than or equal to the routine stained sections. The overall quality of microwave-stained sections was found to be better than the routine stained sections in majority of cases.

Keywords: Microwave staining, routine staining, kitchen microwave oven.

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Introduction

Keeping in pace with the changing scenario towards modernization in the field of medical technology, traditional techniques have been replaced by newer ones. But histotechniques in histopathology more or less still remains the same and have seen few changes. For almost 100yrs, the steps followed to prepare tissues for microscopic evaluation have remained unchanged but the time consumed by these steps have reduced from several days to merely one or two days and now with the advent of microwave tissue processing it has come down to few hours.

We have come a long way from the time the conventional tissue processing was proposed in the 19th century to frozen sections to automatic tissue processor to the successful application of microwaves in the field of histotechniques for fixation and then processing.[1,2] The microwave used for histotechniques works on the principle that electromagnetic field causes excitation of molecules which brings about its rotation. This produces energy in the form of heat from within the materials. This heat enhances the rate of diffusion of fluids in and out of the tissues blocks or sections even more effectively in contrast to conventional heating.[3-5]

However the adoption of microwave irradiation for irradiation of tissue processing had not been realized because of inconsistencies in results, which has been attributed to

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deficiencies in hard ware designs, lack of precise control of parameters such as temperature and power setting. Nevertheless with changing times there have been many methods adopted to overcome these deficiencies of microwave thereby successfully applying it into histotechniques to yield better results without compromises.

The routinely used microwave oven in a histopathology lab is a laboratory microwave oven. This apart from all the advantages it has like maximum output of 2000-3000 Watts, an inbuilt source of adjustable temperature probe, facility for ventilation etc is extremely expensive as compared to a kitchen microwave oven.

Thus the present study is aimed at overcoming the limitations in use of inexpensive model of kitchen microwave oven for laboratory purposes and evaluating the efficacy of kitchen microwave as against the much fancied model of laboratory microwave oven as well as to assess the reliability of the kitchen microwave oven as against the established routine processing and to establish the kitchen microwave as a valuable tool for rapid reporting without compromising on the quality.

The term microwave seems to have first appeared in writing in the first issue of Alta Frequenza[5]. The original magnetron- the main functional unit of microwave was invented at the GE Research Laboratory in 1916. The microwave oven was invented in 1945 by Mr. Percy Spencer for which he was awarded US patent in 1950.[3-5]

Microwaves are non-ionizing electromagnetic waves with frequencies ranging from 300MHz to 300GHz corresponding to the wavelength of 1m to 1mm respectively and all kitchen microwave oven operate at 2.45GHz with corresponding wavelength of 12.2cm.[3-5]

Microwaves works on the same line by causing 'rotation of water molecules wherein one molecule of water has one big atom of oxygen to which, two little hydrogen atoms are attached as shown in figure 1.[3-5] Water molecules have a positively charged side and a negatively charged side, so, when negative charges are brought near electromagnetic field, there is repulsion as they are like charges, causing molecules to rotate, as they are asymmetrical as shown in figure 2.[4] The charges in the electric field are subject to a force from same direction or at 1800 from electric field depending on the sign of the charge.[5] This is true even for dipole of water molecules. Polar molecules are subject to torque in electric field itself. The field itself provides energy for this. Acquired rotational energy is transferred into random motion on collision with other molecules. Oscillating dipoles are hindered by their own inertia

Table 1: The protocol followed for routine processing

Steps	REAGENT/PROCESSING FLUID	TIME
Dehydration	70% isopropyl alcohol	$1^{1/2}$ hr
	80% isopropyl alcohol	$1^{1/2}$ hr
	99% isopropyl alcohol	2 hr
Clearing	Chloroform	Overnight
Impregnation	Molten paraffin	$1^{1/2}$ hr
	Molten paraffin	$1^{1/2}$ hr
	Molten paraffin	$1^{1/2}$ hr
TOTAL TIME- 28-29hrs		

Table 2: The protocol followed for microwave processing

STEP	REAGENT/ PROCESSING FLUID	TIME
Dehydration	99% isopropyl alcohol	30min
	99% isopropyl alcohol	30min
Impregnation	Molten paraffin wax	30min
	Molten paraffin wax	30min
Total time- 2hrs		

Table 3: The protocol followed for H and E staining

REAGENT	TIME
Xylene	10min
99% isopropyl alcohol	10min
80% isopropyl alcohol	10min
Water bath	15min
Haematoxlin	10-15min
Water bath	10min
Acid alcohol	1 dip
Water bath	10min
Eosin	1 dip
99% isopropyl alcohol	1 dip
Xylene	10min

Table 4: The list of criteria, which were taken for evaluation by pathologist

Sl No	CRITERIA	ROUTINE	MICROWAVE
1.	**Tissue architecture** • Fragmentation of tissue sections • Integrity of epithelium and connective tissue.		
2.	**Cellular details** • Size of the cell • Cellular outline clarity • Cytoplasmic details		
3.	**Nuclear details** • Size of the nucleus • Clarity of nucleus • Clarity of nucleoli • Clarity of nuclear membrane • Clarity of chromatin		
4.	**Difficulty in sectioning of tissues** • at 4µm thickness • at 5µm thickness		
5.	**Staining characteristic**		

and by frictional retarding forces from their surroundings. As the molecules slow down in its rotation it causes frictional forces, which produces heat energy. Unlike conventional heating, heating in microwave is from within (internal heating) and its effect occurs throughout the material being irradiated.[3-5]

Materials And Methods

133 tissue blocks from 124 cases received in the Department of Oral Pathology, The Oxford Dental College and Hospital- Bangalore over a span of 1 1/2 year were used in the present study. The equipments used for the study comprised of

1. Processing fluids
   - Isopropyl alcohol (70%, 80% & 99%)
   - Chloroform
2. Paraffin wax
3. Soft tissue microtome
4. Water bath
5. H and E stains
6. Mounting media
7. Glass slides
8. Cover slips
9. Tinseal sheets
10. Glass jars of
    - 500ml capacity for routine processing
    - 150ml capacity for microwave processing
11. Compound Microscopes
12. Basic model of kitchen microwave oven model no. M1739N, which is shown in Figure 3.

Methodology

Each of the Formalin fixed biopsy specimen were cut into approximately two equal halves, of which one bit was sent for microwave processing and the other was sent for microwave processing. The tissues sent for routine processing were processed as per the schedule mentioned in Table 1 and embedded in paraffin wax.

The tissue to be processed in microwave oven were first dabbed on tissue paper to remove excess fixative, the tissues were then wrapped in paper as shown in Figure no 4. In case where multiple bits were to be processed simultaneously, each tissue bit was wrapped individually. Wrapped tissues were then placed in glass jars containing 100ml of 99% isopropyl alcohol as shown in figure 5. The opening of the jars was covered with perforated tinseal sheets. This glass jars was then placed in the outermost circle of the rotating table in microwave oven. Another glass jar of same capacity containing 100ml of tap cold water was placed on the opposite side of the already placed glass jars as a measure of temperature control as shown in Figure 6. The tissues processed in microwave as per the schedule mentioned in Table 2.

Microwave was operated at the lowest output power level of 100 watts. Following impregnation in paraffin wax, the tissue was embedded in paraffin wax using paraffin wax using 'L' blocks smeared with glycerin. Blocks of both routine and microwave processed tissues were placed in refrigerator for 10-20minutes to ensure solidification and easy sectioning. Using semiautomatic soft tissue microtome, the blocks were first trimmed and then sectioned at 5µm thickness. Sections obtained from routine and microwave processed tissues were then mounted separately on 2

Table 5: The statistical values for Tissue Architectur

Tissue architecture	Microwave (n=133)	Routine (n=133)
1. Fragmentation of tissue section (+)	Absent=116 (87.2%) Present=17 (12.8%)	Absent=121 (90.9%) Present=12(9.1%)
2. Integrity of epithelium and connective tissue (+)	Absent=50 (37.6%) Present=83 (62.4%)	Absent=54 (40.6%) Present=79 (59.4%)
Inferences	Maintenance of tissue architecture was found to be similar in both microwave and routine processed tissues with a P=2.67ns	

Table 6: The statistical values of Cellular Details (H and E)

Cellular details in H & E	Microwave	Routine	Significance By student t
Range	5-9	3-9	P<0.001**
Mean ± SD	8.04±1.03	7.09±1.02	
Inference	In H and E stained slides the cellular details of microwave processed tissue were significantly better than the routine processed tissue with p<0.001.		

Table 7: The statistical values of Nuclear Details (H and E)

Nuclear details H & E	Microwave (Mean ± SD)	Routine (Mean ± SD)	Significance By student t
Range	6-15	5-14	P<0.001**
Mean ± SD	12.14±1.96	10.94±1.68	
Inference	In H and E stained slides the nuclear details of microwave processed tissue were significantly better than the routine processed tissue with p<0.001.		

Table 8: The statistical values of staining characteristics (H and E)

Staining characteristics of H and E	Microwave (Mean ± SD)	Routine (Mean ± SD)	Significance By student t
Mean ± SD	2.7238±0.51	2.5±0.58	P<0.001**
Inference	Staining characteristics of microwave processed tissues were found to be better than routine processed tissues with P<0.001		

Table 9: The statistical values of Difficulty in Tissue sectioning

Difficulty in sectioning of Tissue	Microwave (n=133)	Routine (n=133)
1.At 4 µ m	Absent=124 (93.2%) Present=9(6.8%)	Absent=124 (93.2%) Present=9(6.8%)
2. At 5 µm	Absent=130 (97.8%) Present=3 (2.3%)	Absent=130 (97.8%) Present=3 (2.3%)

different slides and then stained simultaneously as shown in Figure 7 with H and E as shown in the Table 3. The mounted slides were assigned a number and coded with two different symbols one for routine and one for microwave processed tissues and the codes were periodically changed to avoid bias. These paired slides were then evaluated by a general pathologist for the criteria as shown in Table 4

The criteria so evaluated were graded as Good =3, Average =2, Poor =1

The results so obtained were later statistically analyzed using Student "t" test.

Results

In this study 133 pairs of slides of which one was routinely processed and the other was microwave processed were stained simultaneously with H and E. The results obtained are as tabulated below.

Tissue architecture was maintained by both routine and microwave processed sections in most of the cases. 12 cases (9.1%) of routine processed sections and 17 cases (12.8%) of microwave processed sections resisted fragmentation of tissue, whereas 79 cases (59.4%) of routine processed sections and 83 cases (62.4%) of microwave processed sections maintained integrity of epithelium and connective tissue as shown in Table 5 and Chart 1.

The cellular details of routine processed sections were graded as average in majority of the cases and microwave processed sections were graded as good in majority of the cases. The microwave processed tissues stained with H and E was better than the routine processed tissue and was statistically significant as shown in Table 6 and Chart 2.

The nuclear details of routine processed sections were graded as average in majority of cases and majority of microwave processed sections were graded as good. The microwave processed tissues stained with H and E was better than the routine processed tissue and was statistically significant as shown in Table 7 and Chart 3.

Staining characteristic of routine processed section was graded as good in 73 cases, whereas 101 cases of microwave processed sections were graded as good. Staining characteristics of microwave processed tissues were found to be better than routine processed tissues with P<0.001 as shown in Table 8 and Chart 4.

Ease of sectioning of tissues was seen in 93.2% of cases at 4µm and in 97.8% of cases at 5µm. This was found to be identical in both routine and microwave processed tissue blocks as shown in Table 9 and Chart 5.

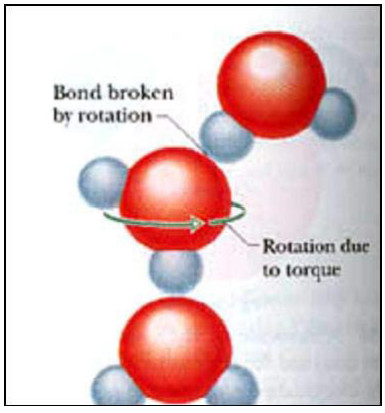

Fig.1: Asymmetric water molecules a large atom of oxygen & 2 small atoms of hydrogen (Courtesy source- Physics hot pockets accessed on 20th May 2009 https://scienceblogs.com/startswithabang/)

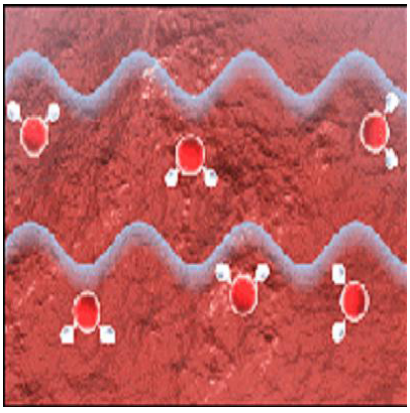

Fig. 2: Water molecules rotating after repulsion (Courtesy source- Physics hot pockets accessed on 20th May 2009 https://scienceblogs.com/startswithabang/)

Fig. 3: Basic model of kitchen microwave oven used in this study (Samsung; Model no. M1739N)

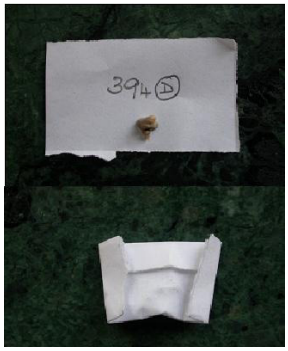

Fig. 4: Wrapping of tissues in a paper for microwave processing.

Fig. 5: Tissue wrapped tissue placed in a glass jar containing 99% isopropyl alcohol.

Fig. 6: Two glass jars of the same capacity placed in the outermost rotating table of the microwave of which one contains tissue to be processed with alcohol and the other jar containing tap water.

Fig. 7: Tissue sections of routine and microwave processed tissue stained simultaneously.

The time consumed for routine processing was 28hrs whereas microwave processing consumed 2hrs. The total amount of alcohol consumed throughout the study by routine processing was approximately 7 liters of isopropyl alcohol whereas microwave processed consumed 10 liters of isopropyl alcohol. Routinely processed tissues showed shrinkage of 2mm on an average whereas microwave processed tissues showed shrinkage of 3mm on an average.

Discussion

The new technique of processing tissue using a microwave employed in this study represents a major change from conventional tissue processing. The ease of application and speed of this technique has significantly reduced turnaround time in diagnostic labs for the past 3 decades. Initially application of microwave techniques into histotechnology was not accepted but nowadays is growing in its popularity and versatility.

Literature from Physics and chemistry suggest that the viscosity of liquid decreases at constant pressure and absolute temperature thereby increasing diffusion and heat is known to increase diffusion. Heat has known to increase diffusion and so initially conventional heating was employed into histoprocessing in order to achieve increased diffusion thereby reducing the processing time. But this led to uneven distribution of heat energy, which resulted in hardening of outer layer whereas the central part remained unprocessed and therefore soft. [3-7]

The kitchen microwave oven used in our study had a maximum output of 800Watts and we operated the microwave at the lowest output of 100Watts throughout the study. Although literature from various studies suggests that the microwave can be operated at higher output levels i.e., 200 Watts to 200Watts in turn reducing the time of processing from 1-2hrs to as less as 5 min, we preferred to opt for the lowest output of 100Watts in order to reduce or avoid damage to tissues by the heat.[8]

As the exact temperature at which the microwave was operated could not be assessed we decided to take measures for temperature control by water load as mentioned in a review by Gary R Login.[9] However, these measures were not standardized in our study and were achieved with the help of glass jars with tap cold water of the same capacity as that used for tissue processing.

Of the 133 pairs of H and E stained slides, maintenance of tissue architecture was found to be almost similar in both routine and microwave processed tissues. This is similar to the findings in the study of Azorides Morales.[10]

The cellular details and the staining characteristics of the microwave processed tissue were found to be better in comparison to the routine processed tissue. This is consistent with the studies of Kok and Boon and we agree with their conjecture that the superior results with the microwave method are due to the uniform distribution of the heat, which causes similar uniform effect of alcohol on the tissue.[2]

The nuclear details of the microwave processed tissues were found to be better in comparison to the routine processed tissues, except for clarity of chromatin and clarity of the nuclear membrane which were similar in both routine and microwave processed tissues. We have not come across any literature that has emphasized on these findings, so we presume that as chromatin and nuclear membrane are not usually seen in all the cases under the light microscopy and were not of any diagnostic significance and hence not an effective parameter in our study.

Ease of sectioning of tissues was present in almost all the case but only 5 tissues posed difficulty while sectioning at 5µm of which 3 were cases of fibrous tissue 1 was inadequately fixed 1 was a necrotic tissue. 10 cases were tough to section at 4µm; it included 5 cases of fibrous hyperplasia in addition to the above cases. To the best of our knowledge we have not come across any study, which have emphasized on this finding.

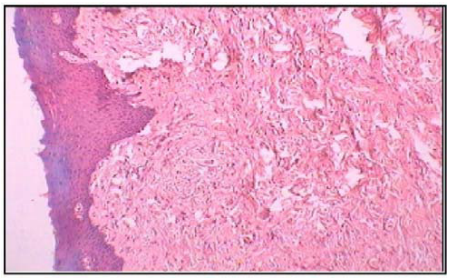

Fig. 8A: Tissue architecture in routine processed. (H and E,10X)

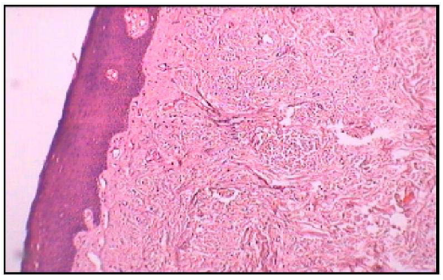

Fig. 8B: Tissue architecture in microwave processed. (H and E,10X)

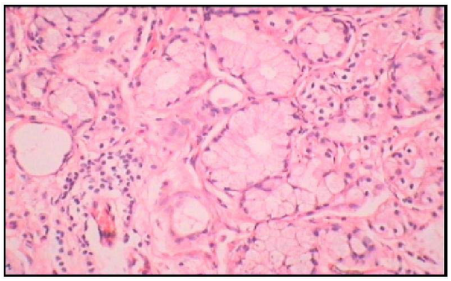

Fig. 9A - Cellular and nuclear details in routine processed. (H and E, 40X)

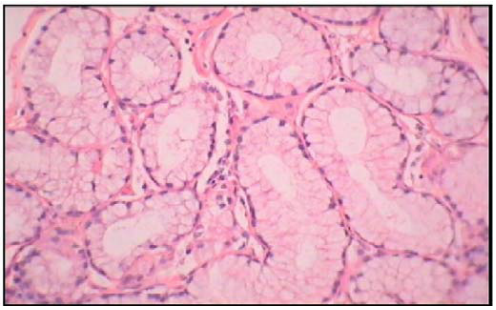

Fig. 9B: Cellular and nuclear details in microwave processed. (H and E, 40X)

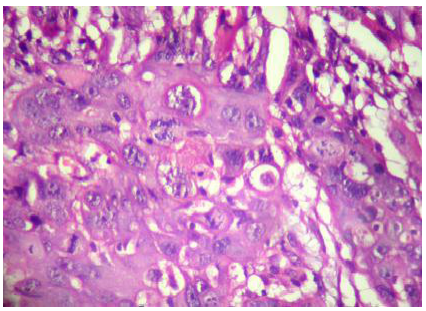

Fig. 10A: Staining characteristics in routine processed. (H and E, 40X)

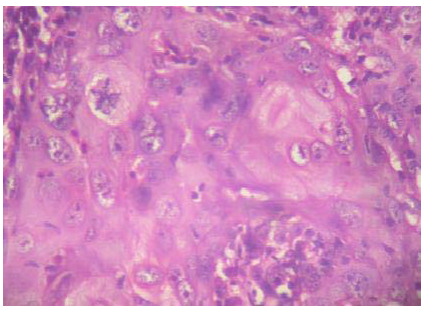

Fig. 10B: Staining characteristics in microwave processed. (H and E, 40X)

Metal causes sparking and plastic melts inside microwave so neither metal cassette nor plastic cassettes can be used in microwave, Teflon coated cassettes are the choice to be used in microwave. But they are expensive, so we opted to wrap the tissues in paper, which are much cheaper and easily available as a means to prevent settling of tissues in the bottom. Tissues were dabbed in tissue paper after their removal from fixative and wrapped in paper and then placed in alcohol jars.

The maximum distribution of microwave power is seen in the outermost ring of the rotating table 9. So we placed the glass jars with processing fluids and water load in these rear corners, opposite to each other throughout the study. Two dehydration cycles was sufficient for most of the cases in this study. But almost 15 cases had to be reprocessed i.e., they required an additional dehydration cycle. Two cycles of impregnation was found to be satisfactory for all the tissues.

A maximum of 5 individually wrapped tissues could be placed in each container with alcohol. If more than 5 tissues have to be processed then two containers with 100ml alcohol in each were used. The tissue would be equally divided (not more than 5 in a bottle) and processed. In such situation the beaker containing tap cold water (used to counteract excess heat) was omitted as the temperature is controlled by the other beaker containing tissue bits and alcohol.

It was noted that multiple bits when processed simultaneously consumed less alcohol than tissue bit processed individually in a microwave i.e., when single tissue was processed we noted that at the end of first dehydration cycle (end of 30min) the alcohol remaining was more than half and at the end of second dehydration cycle more than 15-20ml of alcohol was remaining back. But when multiple bits of tissues were processed simultaneously, at the end of dehydration (after 1hour) 30-40ml of solution remained back. This finding was consistently noted in majority of the cases.

In terms of cost effectiveness, routine processing appears to be more costly than microwave processing. In routine processing in addition to isopropyl alcohol, chloroform was used as a clearing agent and the tissues were impregnated in wax bath operated at more than 100Watts for 4 1/2hrs whereas in microwave processing though it consumed more isopropyl alcohol, it did not require any intermedium and hence chloroform was completely omitted in microwave processing. The total time or irradiation of tissues was for a total of 2hrs at 100Watts including dehydration and impregnation in paraffin wax.

Both the microwave and routine processed tissues showed some amount of shrinkage. The overall shrinkage of microwave processed tissue was <3mm whereas routine processed tissues showed shrinkage of <2mm. The shrinkage noted in both routine and microwave processed tissues is due to the shrinking effects of alcohol where there is replacement of water molecules from the hydrophilic sites of the peptides chains in the denatured proteins. The shrinkage noted is negligible and did not interfere with the diagnosis; the slight increase in shrinkage in microwave processed tissues could be due to the heat used in the study.

Prasad G Kango 2011 processed a variety of tissue specimens

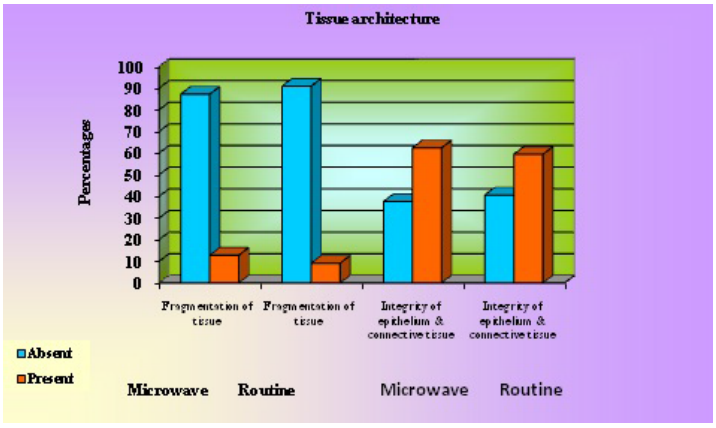

Chart 1: Comparison of tissue architecture between routine and microwave processed tissues sections stained with H and E.

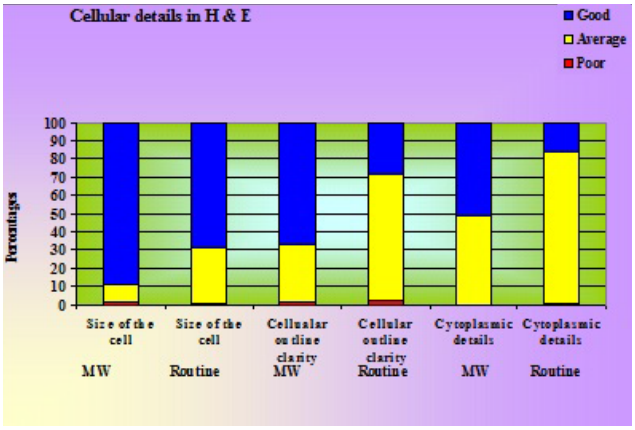

Chart 2: Comparison of cellular details between routine and microwave processed tissues sections stained with H and E.

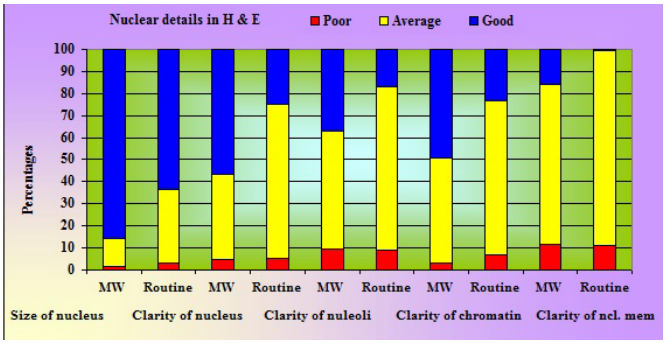

Chart 3: Comparison of nuclear details between routine and microwave processed tissues sections stained with H and E.

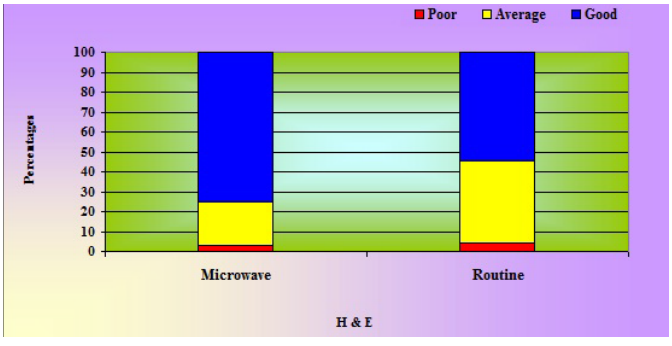

Chart 4: Comparison of staining characteristics details between routine and microwave processed tissues sections stained with H and E.

like benign tumors, reactive lesions, precancerous and malignant lesions using a kitchen microwave oven. They found that the microwave technique showed no alteration in tissue architecture, cellular and nuclear morphology and staining characteristics of cells.[11]

Studies by Harsh Kumar 2014 have further reduced the time of processing by reducing the time of clearing as well as paraffin impregnation and found that there was no compromise in the quality of tissue sections.[12]

Mahesh Rao 2020 fixed, processed and stained tissue sections of different thickness by using kitchen microwave and compared it against conventional method. They found that microwave technique reduces the time in the lab along with good cellular, nuclear clarity of sections from both techniques. However the results of tissue sections >9mm was noted to be inadequate.[13]

In our study we found that the overall quality of microscopic tissues of microwave processed tissues was found to be better than routine processed tissues in most of the cases. Following the success of our study we have employed microwave processing as an adjunct to routine processing especially when urgent reporting of specimens is required on day-to-day basis.

Conclusion

Microwaves, a form of radar-wave-induced heat when applied in histotechniques reproducibly yields histolologic material of similar or superior quality to that provided by traditional conventional processing. When used properly microwave processing offers advantages including less time consumption, expediency, and safety potential for preservation of integrity of specimen and improves the workflow in the laboratory, permitting the preparation of the diagnostic material, hence today microwave finds its application into fixation, processing, accelerating routine stains, special, metallic as well as immunofluorescent, immunohistochemical stains for both light and electron microscopy. We noted in our study that overall quality of microscopic tissues of microwave processed tissue were found to be better than routine processed tissues without compromising on the quality and well economically beneficial, especially when the tissues are received with request for urgent reports. Based on the findings of our study, we hypothesize that better quality of microscopic images can in fact be obtained with the use of kitchen microwave oven with adequate temperature control methods.

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