DISCUSSION

To meet the prerequisites of clinicians who are treating critically ill patients, it is of utmost desirable to process the histopathological biopsy specimen immediately. Conventional methods for tissue processing require 16 to 24 hours, which delays the treatment. Tissue processing by microwave method reduces the duration of cycle and permit faster processing and staining of histopathologic tissue.

There are different data that compare quality of routine processed tissue and microwave processed tissue. In current study, 350 paired tissues (total 700) were randomly selected. One part was processed and stained by routine method and another part was processed and stained by the microwave method. Then, both the tissues were compared for quality of histologic microscopic slides in a blinded manner.

On analysis of results, microwave processing and staining showed significant reduction of time of preparation for histological sections with obvious preservation of section quality or "readability."

Turnaround time is a vital issue since a number of years and also going to be more important during these years of patient care and commitments to lessen the overall cost of health care services.

The modification of the conventional technique for preparation of tissue by the well accepted overnight tissue processing method to microwave assisted tissue processing method might significantly decreases the turnaround time. This allows early diagnosis which would help for satisfactory management of the patient. Thus, reduction in turn around time reduces patient and clinician dissatisfaction and increases patient compliance as patients with critical illness or neoplastic diseases can be diagnosed early and therapy can be initiated.

Table 7.1 Comparison of Turn around time of various studies:

Name of study	Processing time (Routine method)	Processing time (Microwave method)	Staining time (Routine method)	Processing time (Microwave method)
N Amrutha et al[70]	7 hour	2 hour	31 minutes 20 seconds	16 minutes 45 seconds
ShankarGouda Patil et al[71]	7 hours	2 hours	31 min 20 sec	16 min 45 sec
Mahesh Babu[4]	270 minutes	42 minutes	40 minutes	33 minutes
Anamika Sinha et al[72]	17 hours	35 min 25 sec	-	-
Anita Choudahury[73]	11-12 hours	15 minutes	-	-
Kartesh Singla et al[19]	7 hour	1 hour	-	-
Suyog Tupsakhare[74]	29 hour	1 hour 5 minutes	-	-
Harshkumar et al[48]	18 hour 30 minutes	55 minutes	-	-
Anil Pandey[75]	28 hour	2 hour	-	-
Rajat Nangia[76]	48 hours	1 hour	-	-
B.S. Shruthi et al[22]	60 minutes	15 minutes	-	-
Promil Jain[77]	960 minutes	8.5 minutes	-	-
Ralph Rohr[13]	8 hour (small biopsy)	15 minutes	-	-
	12 hour (large biopsy)	60 minutes	-	-
Bhuvanamha Devi et al[78]	7 hour	1 hour 22 minutes to 2 hour 22 minutes	-	-
Archana Mukunda [51]	-	-	1 hour 15 minutes	20 minutes
Present study	16 hour	67 minutes	20 minutes	14 minutes

Many researchers have studied the methods and effects of microwave assisted tissue processing and staining. The objective of the current study is to determine whether microwave assisted technique is comparable better than standard overnight routine processing technique for tissue preparation.

Morales et al[50] studied the results of a fully automatic microwave assisted rapid tissue processor (RTP) for histopathologic evaluation of tissues in association with turnaround time for reports of histopathology tissues. Also, the comparison was done of histopathology outcomes by this method and those obtained from the conventional overnight tissue processing (CTP) method. It was done blindly by 9 pathologists having varying years of experience. The comparison was done for histopathology reports turnaround times for the duration of 1 year between RTP and the previous year for CTP. The conclusion of study was that the histologic material produced by RTP method was of parallel quality to CTP. There is marked improvement of turnaround time for histopathological reports and, especially, reporting on same day could be attained in nearly 55% cases as compared to lesser than 1% before usage of the RTP. In addition, RTP method augments safety by removing harmful chemicals like formalin and xylene.

Visinoni F et al[79] in 1998 described a newer technique for ultra-fast histoprocessing which decreased the tissue processing time for needle and endoscopic biopsies to 30 min and other biopsy specimen up to 4 mm thickness to 120 min. The processor had microwave irradiation having accurate computer control of power, time, temperature, vacuum, and pressure. The tissues which were processed by this method demonstrated optimum cytomorphology and improvement in properties of tissue cutting. In addition, staining properties was also optimum without detrimental effects on routine staining, histochemistry, or immunohistochemistry. This newer processing method proves that it had brought a major beneficial change in conventional tissue processing and eliminated the usage of harmful solutions like xylene. Therefore, this easy and speedy procedure can markedly reduces turnaround times in diagnostic laboratories.

Ralph Rohr[13] examined randomly selected 158 paired samples. In vacuum tissue processor, one sample of each pair was processed by two different schedules for small and large biopsy by routine overnight method. Other part of the pair was processed as per the two different microwave based schedules, a shorter schedule for smaller biopsy of less than 2 mm thickness and 10 mm diameter, and a longer schedule for biopsy larger than 2 mm thickness or having large amounts of blood, mucus or both. In temperature-controlled microwave processor, the microwave schedule was

executed. The total duration of microwave processing times were 15 minutes and 60 minutes for smaller and larger biopsies respectively. These times are well compared to routine 8 hours processing time for smaller biopsies and 12 hours processing time for larger and fatty biopsies. The study concluded that there was positive effect on turnaround time in microwave method and the excellence of microscopic slides from the routine processing and the microwave-processing methods were extremely equivalent. It was not possible to differentiate between the 2 methods by examining the tissue sections. In present study, there is significant amount of time saving in microwave method that takes 67 minutes to process as opposed to the overnight procedures that is routinely used which takes 16 hours. Hence, its use can be given priority for whom an immediate histopathological diagnosis is needed for initiating life-saving antimicrobial, immunosuppressant or chemotherapeutic intervention.

Various studies have also proved that microwave assisted tissue processing is a method of a quick delivery of tissue sections. The quality of the microscopic tissues is similar to that of sections prepared by conventional method. [9,18,71]

The tissues processed by both methods were examined and assessed for change in colour, consistency and shrinkage. The colour and consistency (at 58^0C) were consistent as before processing while minimal shrinkage was identified in microwave processed tissue. The study by Mahesh Babu et al[4] used reagents identical to present study. The study examined dimensions of tissue in terms of volume and considered reduction in volumes as shrinkage. A 4% excess shrinkage as compared to routine processing was experienced. Here it is explained that it could be due to the heat generated by microwave oven. Present study also shows excess shrinkage of 5.8% by microwave method. However, tissue shrinkage was not observed by Kango and Deshmukh[10], Kartesh Singla et al[19], Morales et al[50] et al. It could be due to different protocols and reagents has been used by these various studies.

Shankargouda Patil et al[71] evaluated mean score for clarity, cytoplasmic details, nuclear details and colour intensity on epithelium, muscle, adipose tissue and glandular tissues. Total 40 tissues were compared by microwave processing and staining method with conventional processing and staining method. The p value was

0.066 indicates that between the two methods, there was not any statistical difference. In present study, clarity of cellular details and nuclear membrane were equal by both methods.

Kartesh Singla et al[19] compared microwave, conventional and rapid manual technique. The histopathological evaluation was done for cytoplasmic and nuclear details and graded as distinct and indistinct. The study determined that nuclear cytoplasmic contrast was good, cellular outline was distinct and colour intensity showed crisper and good contrast by microwave method followed by conventional tissue processing and rapid manual processing techniques.

Harsh Kumar et al[48] evaluated total 150 specimens (total 300 tissues) for cellular morphology and nuclear morphology by both conventional and microwave techniques. Grade 1 was given when there was distinct cell and nuclear morphology and grade 0 was given when there was indistinct morphology. There was not any significant statistical differences observed for cell morphology (p=0.472) and nuclear morphology(p=0.552) in the products obtained by two methods.

N Amrutha et al[70] evaluated 30 randomly picked tissue samples. Each sample was cut into two equal parts (a total of 60 tissues). Processing and staining of one part was by routine method and both the procedures of other half was done by microwave method. The results were evaluated blindly by four observers. The histopathological evaluation for cellular clarity, nuclear details, cytoplasmic and colour intensity was done. The results obtained were better in microwave processed and stained tissues than conventional protocol. The p value was 0.068. It indicates there was not any significant statistical difference between the two methods. In addition, kappa statistics was 0.900, that exhibited greater consistency of results between the two observers.

Rajat Nangia et al[76] examined 20 oral mucosal biopsy specimens. They were cut into three equal parts and each processed by routine, domestic microwave and commercially available laboratory microwave techniques. Parameters studied were colour intensity, uniformity of staining and nuclear and cytoplasmic details. The results were slightly better in laboratory microwave method followed by domestic microwave method and routine method. P values were statistically insignificant

for all parameters except colour intensity. Present study also evaluated for former two methods of 350 specimens for various parameters.

Bhuvanamha Devi R et al[78] studied 135 paired tissues sections. The 10 Paired tissues were processed by a conventional and microwave method. Later, the number of tissues to be processed were gradually increased to B15, C20, D25, E30 and F35.Reagent quantity used to ensure that tissue was submerged within it. The tissues which were processed by both methods were evaluated by blind study, that was independently examined by two observers. The qualities of slides were evaluated, based on 4 parameters: cytoplasmic details, nuclear details, architecture of tissue and characteristics of staining. The study concluded that, when the maximum number of samples within the microwave oven was of 25 samples, the morphological outcomes (quality) of the domestic microwave processed tissues were comparable to the conventional methods.

Kango and Deshmukh et al[10] processed 50 specimen (total 100 tissues) and compared histopathological examination of slides processed by microwave and conventional methods. The sections were evaluated for cellular morphology, nuclear morphology and staining characteristics. The slides were examined by four experienced pathologists. The alpha values of all observers in percentage, were 75% in concordance for cell morphology, 60% for nuclear morphology and 56% for quality of staining. The study results showed that microscopic quality of the sections of microwave processed tissues were analogous to or fairly better than tissue processed by conventional method with the equal time of formalin fixation. Therefore, rapid microwave-assisted tissue processing is the ideal method to produce better quality sections. In addition, the best microscopic sections prepared by the method showed no differences in the cellular and nuclear morphology in few tissue types.

Raju Shashidara et al[49] studied kitchen microwave assisted accelerated method for tissue fixation and processing of oral mucosal biopsies. The parameters included for evaluation were diagnostic evaluation of tissues, section quality, cellular outline, nuclear details, staining quality and occurrence of artifacts (section folds, heat artifacts). In comparison, large numbers of samples fixed and processed in microwave were classified as optimal while all parameters were tested without the

occurrence of artifacts by routine fixation. However, there was not any statistically significant difference in the examined parameters.

T Mahesh Babu et al[4] studied 15 oral mucosal biopsies processed by both conventional and microwave methods and later stained H & E by conventional and microwave methods. They were evaluated blindly by six observers. The parameters examined were cellular clarity, cytoplasmic details, nuclear details, color intensity, epithelial and connective tissue interface including fibrous tissue. The overall quality of slides prepared by microwave assisted methods of processing and staining were comparatively better than the slides prepared by routine methods of processing and staining. The entire processing time involved in microwave and conventional method were 42 minutes and 270 minutes respectively. Also, the duration of H and E staining by microwave and conventional method were 33 minutes and 40 minutes respectively. The study concluded that microwave technique of tissue processing can be adopted in the regular histopathology laboratory on a regular basis, and considering the considerable shortening of time period, microwave technique can replace the well-established routine tissue processing.

Alka Mary Mathai et al[9] compared one hundred and eighty-five twin tissue sections from various organs. The comparison of quality of histopathology slides was done between microwave and conventional histoprocessing methods. The study also determined its beneficial effect on turnaround times and decrease tissue processing cost. Each tissue sections were divided in two parts; first was considered as experimental group and another as control group. The experimental group tissues were again divided into six groups and processed in a vacuum microwave as per the six protocols that is I to VI. While control group tissues were processed by the conventional method and compared. Finally, the microscopic tissue quality obtained by these two methods was similar. Microwave processing decreased the processing time with preservation of overall quality of the histologic section and was cheaper too. In addition, the microwave method did not yield any deleterious effects on special stains.

Pritam Panja et al[12] compared ten different tissue samples and each sections were cut into three pieces. These sections were processed by three different methods of tissue processing namely routine manual method, rapid manual method and microwave

method. The study did not show statistically significant difference in the staining quality, clarity of nucleus cytoplasmic differentiation of epithelial tissue, fibrous tissue, glandular tissues as well as presence of artifacts. In addition, the study also proves that microwave tissue processing was more cost-effective than other methods.

L. Ralph Rohr et al[13] included 158 paired specimens from 111 patients in their study. One part was processed by routine method and another half was processed by the rapid laboratory microwave technique. Later, comparison of the slides were done for the quality of histologic preparation blindly by 2 pathologists. On examination, study concluded that, the quality of microscopic tissues from the routine processing and the microwave-processing methods were very similar. It was impossible to differentiate between the 2 techniques by the study of the tissue sections.

Surg Capt P Sivadas et al[39] studied 200 tissues (100 biopsy and 100 necropsy tissues) for microwave assisted fixation and rapid processing by three different microwave protocols with conventional methods. The tissues were taken in three different sizes, 1x1x1 cm, 1x1x0.5 cm and 0.5x0.5x0.5 cm. for obtaining high quality paraffin sections, A rapid method is described that allows paraffin blocks preparation in approximately 20 minutes which was microwave assisted tissue processing method. Tissue blocks of size 1x1x0.5 were found to be ideal for processing. Three schedules were assessed for tissue processing by using different timings of exposure to Increasing concentrations of alcohols as well as chloroform and wax. It was noted that an addition of a step of exposure to 70 % alcohol in protocol 3 as a first step of tissue dehydration led to prepare paraffin blocks that were evaluated as excellent by light microscopically and significantly similar to conventional methods.

Promil Jain et al[77] conducted a study to compare the time duration and histopathologic section quality of prostatic tissue processed by rapid microwave and conventional techniques using morphometry. The study included paired fifty prostatectomy specimens of four to five mm thickness. A part of the pair was processed overnight by conventional tissue processing and another part by microwave tissue processing. Time taken were compared for processing by both techniques which showed that time taken for dehydration, clearing and impregnation in microwave technique was of lesser as comparison to histoprocessing performed by

conventional technique. Morphometric analysis was done on prostatic tissue slides processed by conventional and microwave techniques. Morphology, that is staining patterns of prostatic tissue that were processed in minutes by microwave method were similar to the sections that were processed in days by conventional method. The study concluded that domestic microwave could be used for histoprocessing to speed up the processing with preservation of morphology of tissue and is cost effective than laboratory microwave, that is commercially available. In addition, the processing time was markedly decreased from days to minutes.

Archana Mukunda et al [51] compared the reliability of modified kitchen microwave staining technique with routine staining technique. Sixty paraffin blocks of tissues were used to prepare 20 pairs of slides to apply 4 different stains including hematoxylin and eosin, 0.1% toluidine blue, Van Gieson's and periodic acid-Schiff. By routine method, one slide from each tissue was stained while another slide stained inside the microwave. Microwave staining significantly reduces the staining time from hours to seconds. Also, the cellular and nuclear details as well as staining characteristics of microwave stained tissues were of improved or equivalent quality to that of routine stained tissue. The overall quality of microwave-stained sections was also better than the routine stained tissue in most cases.

Similarly a new method of Ziehl-Neelsen stain by microwave oven was developed by S. Hafiz et al [15] for the step of heating. The smears were covered with carbolic magenta and it was irradiated by microwaves for 30 seconds at maximum power of 640 Watts. The staining of tissue sections by microwave method with carbolic magenta was compared to the results obtained by the conventional Ziehl-Neelsen method. The benefits of microwave irradiation method was that the smears have clean background and absence of crystalline deposits. In addition, processing was very fast because the duration of procedure was decreased by 15 minutes for each lot. The flame was not required, so there was no risk of the slides cracking due to direct heat and reduces risk of fire due to naked flames. In addition, safety of technicians was ensured as mycobacteria were destroyed by microwave irradiation for 30-60 seconds. The characteristics of decolourisation remained similar and a specific benefit for staining histological sections was that the laborious stage of dewaxing was removed.

Godwin O. Avwioro[40] compared staining quality of microwave processed and conventional processed tissues. Normal lung, kidney, liver, intestine and heart tissues were dissected from an adult Wistar albino rat. Then, the tissues were fixed in 10% formol saline and processed by the conventional method. The pair of specimens were prepared, out of that one part was processed by conventional method and other part by microwave method. Section cutting done by the rotary microtome and later stained by heamatoxylin and eosin stain for general tissue structure examination. Moreover, Verhoeff's van Gieson iron haematoxylin for elastic fibres, Weigert's van Gieson iron haematoxylin for collagen fibres, PAS (periodic acid Schiff) stain for neutral mucopolysaccharides, Gordon and Sweet's method for reticular fibres, Alcian blue (pH2.5) for carboxylated and sulphated mucopolysaccharides, Congo red stain for amyloid, Masson's trichrome for collagen and muscle fibres and Gomori's aldehyde fuchsin for elastic fibres were performed. In Microscopic examination, there was no significant difference in the staining of routine method in comparison to the microwave processed tissues. The study concluded that nuclear, cytoplasmic, extracellular and intracellular details appeared to be similar by both methods.

Hendrik E. Moorlag et al[41] performed study by modification Perl's, Alcian blue, Fontana-Masson and Romanowsky-Giemsa stain by means of microwave irradiation. It was observed that modification of the staining technique evidenced as very fruitful in routine practice for individual cases where immediate diagnosis is needed. By applying this method, the total time duration for staining is a matter of maximum for few minutes. Results achieved were very good and consistent. The method was cost wise beneficial as only few drops of staining solutions were utilized.

Lyska L. Emerson et al[80] studied whether the quality of immunohistochemical staining was affected by microwave assisted rapid tissue processing. There was total thirty specimens (twenty neoplastic and ten nonneoplastic) were selected from regular histopathology work. Pair of tissue blocks were prepared from the specimen; one part of pair was processed by rapid microwave processing method and another by routine tissue processing method. From the blocks, two microarrays of 60 punches were prepared. The microarray blocks were assessed for staining intensity and extent of staining by performed by 44 routinely used immunohistochemistry antibodies. Independent review of slides was done by two pathologists blindly, that is

without knowing the method of tissue processing. High level of compliance in quality was found in 5,280 tissue punches assessed. Between microwave and routinely processed methods, intensity and extent of immunohistochemical staining were compared. Therefore, the research showed that quality of immunohistochemical staining is equivalent between rapid microwave and conventional tissue processing method. Therefore, immunohistochemical analysis is not contraindicated if tissue processing was performed by rapid tissue processing by microwave method.

Nitin Gangane et al [61] determined whether cell blocks prepared from residues of sample by microwave method would aid for diagnostic precision. After preparation of cervical smears, The residual material left on the spatula was dipped in ethanol. They were used to make microwave assisted preparation of cell blocks of 260 patients. Papanicolaou (Pap) smears and cell block sections were assessed independently. Sensitivity and specificity of both the techniques were calculated for examination of malignancy and intraepithelial lesions. Sensitivity of cytology smear for detection of malignancy was 79.16% and the specificity was 100%. While sensitivity of cell block was 86.3% and specificity was 100%. The quality of the section and staining by microwave method was very good. In addition, if further modification of technique by microwave fixation and processing significantly decreased the turn around time. Cell blocks made from residual cervico-vaginal smears from the sample can aid the accuracy of diagnosis of Pap smear. Thus, patients did not need to suffer another supplementary procedure.

Maniyan Prakash Sumitha et al[62] processed 80 cell blocks in domestic microwave oven and compared outcomes with FNA smears. FNA material rinsed in Phosphate buffer solution and centrifuged to form clot. This was followed by addition of plasma and afterwards thromboplastin. The clot was taken on Whatman filter paper and processed in microwave oven with short processing cycle such that cell block is processed in less than 10 minutes. The cases were processed in such a way that FNA smears and cell block were available on same day and almost at same time of examination. In addition, ancillary tests like IHC can be done in indicated cases as well as cell block could be compared with respective histopathology sections whenever available.

A Kennedy et al[21] studied the quality of microscopic image of cryostat sections that had been prepared by microwave assisted fixation. It was compared to conventional air drying of the tissue sections. The rapid microwave assisted special stains such as Periodic acid Schiff, Alcian blue, Gordon and Sweets's reticulin, Masson Fontana, Elastica, Prussian blue and Van Gieson were performed within three minutes of cutting a cryostat section. The cytological detail of nuclei was very clear using a microwave method, allowing more precise determination of cell type. Therefore, they concluded that the microwave appears to have great potential for improvement of the diagnostic accuracy of frozen section procedure.

Thus, microwaves can be used for tissue processing and staining without affecting architecture of cells. There is also significant shortening of turn around time. It is a blessing for pathologist as whenever immediate diagnosis is needed by clinician to meet the needs of critically ill patients, this method comes for rescue. It is heartening to see the growth of this very useful technology in our medical sector.