TISSUE PROCESSING TECHNIQUES: A COMPARATIVE STUDY

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ABSTRACT

Background: Conventional tissue processing of histologic specimens has been carried out for many years, which is a time-consuming method resulting in 1-day delay in diagnosis. The time honored conventional method of tissue processing, is now seeing the trend of being replaced, owing to fulfill the needs of clinicians for rapid diagnosis. The most commonly employed means of tissue processing are conventional tissue processing, rapid tissue processing, microwave tissue processing and automatic tissue processing.

Aim: The present study aims to compare the different tissue processing techniques- conventional, rapid conventional, microwave and automatic tissue processor in terms of the effect on staining, cytoplasmic-nuclear details and tissue shrinkage. The time factor involved in the various processes is also assessed.

Methodology: A total of 40 biopsied tissue samples were included in the study. The specimens were fixed in 10% formalin for 24 hours to ensure adequate fixation. The specimens were then subsequently photographed and cut into four equal halves to be processed by conventional, rapid conventional, microwave and automatic tissue processor. The sections obtained by the above processing techniques were stained by haematoxylin and eosin stain, and were subjected to a blind study. Each slide was evaluated by four independent observers and were graded into excellent, good, average and poor, based on the cytoplasmic and nuclear morphology and texture of the tissue of the stained sections.

Results: The results of the study found that there was a statistically highly significant difference in the quality of tissue staining processed in the different types of tissue processors. There was no statistical significant difference in the interobserver opinion on the cytoplasmic- nuclear morphology and tissue texture of the stained sections obtained by the different processing techniques.

Conclusion: The study concluded that, good quality of tissue sections could be obtained by conventional tissue processing technique when compared to the other processing techniques. Microwave processing technique has a positive impact on the turnaround time, permitting same day diagnosis of the biopsy tissue specimens.

KeyWords: *Microwave method, rapid method, routine method, tissue processing.*

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INTRODUCTION

Histology is a word derived from Greek language which means study of tissue and its architecture.[1] Histological study basically involves preparation of tissue for microscopic observations. Tissue processing is a biological procedure carried out in pathology departments, for the purpose of studying the microscopic structure of tissues through identification of morphological changes, to observe characteristics of certain diseases under a microscope. Bancroft and Gamble (1995) proposed tissue processing as a method in which tissues are preserved by manual or automatic means to retain their life like state and cellular components for further analysis by pathologists.[2] The whole course of tissue processing for the basis of tissue diagnostics is referred to as histopathology. It is a means of tissue visualization on a cellular level, it is a fundamental tool in the study of diseases.

Biopsied tissue sample from the patient is subjected to a series of steps, to enable for final microscopic observations of the sections which are of diagnostic quality. Every step in the processing of tissue is of paramount importance, beginning from procurement of the specimen to the final staining and diagnosis.[3]

Tissues must be adequately supported before they can be sectioned for microscopical examination. Tissue processing employs the stages of fixation, dehydration, clearing, impregnation and embedding; each of a designated duration to ensure completion of procedure.[4] Whilst they may be sectioned following a range of preparatory methods, tissues are more commonly taken through a series of reagents and finally infiltrated and embedded in a stable medium which when hard, provides the necessary support for microtomy. Advent of newer technology and instrumentation have increased the role of the histology laboratories in early delivery of diagnostic care to the patients.[5]

Routine manual tissue processing has been the most commonly employed method for the past 100 years.[6] It includes the aforementioned steps and is completed in 21-24h. Its advantages are its reliability and its inexpensive nature. The disadvantage is that it is a time consuming procedure.[5]

Rapid manual tissue processing is of a shorter duration than the above method, requiring 3-4h. It includes the same steps as in routine method, but for shorter durations. The advantages are that it consumes only 20% of time as compared to routine method and the disadvantage is that noxious chemicals like xylene and formalin need to be used and there is a greater degree of tissue distortion and shrinkage.[3]

Microwave method is a recent tissue processing technique, first used by Boon and Kok in 1985.[7] In this process, the penetrative properties of the microwave and the conversion of this incident energy into heat, is made use of, the advantages include shorter processing times, eliminating noxious chemicals like xylene and lesser degree of denaturation of nucleic acids.[8] The disadvantages are the high costs involved. The advent of microwave tissue processing has considerably influenced the tissue processing from few days to few hours.

Automated tissue processors are a comparatively newer innovation in tissue processing technique which has offered wide choice of programming of tissue processing automatically eliminating human errors.[9]

Newer techniques are getting introduced with the demand for faster or early reporting, such as rapid manual and microwave processing. Each of them is unique with their own set of advantages and disadvantages. Turn-around time has been an important issue for many years and has become increasingly important in this age of managed care and commitments to overall reduction of costs for health care services.[10]

Tissue processing for years is carried out by the conventional method, which is a time-consuming technique resulting in 1-day delay in diagnosis.[11] However, in this area of modernization and managed care, rapid diagnosis is increasingly desirable to fulfill the needs of clinicians.

Considering these facts, the four different methods of tissue processing- conventional, rapid conventional, microwave and automatic tissue processor were compared in the present study in terms of the effect on staining, cytoplasmic-nuclear details and tissue shrinkage. The time factor involved in the various processes was also assessed.

AIM:

The aim of the present study was to compare different tissue processing techniques- conventional, rapid conventional, microwave and automatic tissue processor.

OBJECTIVES:

1. To compare tissue sections processed by conventional, rapid conventional, microwave and automatic tissue processor.
2. To evaluate the reliability and time factor of the microwave with that of automatic tissue processing and routine processing and rapid conventional methods.
3. To assess the impact of all the four process on staining quality of the sections.

METHODOLOGY

Source of data:

The study was conducted on oral soft tissue biopsy specimens which were received for diagnostic purposes to the department.

Sample size and method of collecting data:

The study group consisted of 40 biopsied tissue samples. The specimens were fixed in 10% formalin for 24 hours to ensure adequate fixation. Their gross features, such as weight, color, consistency, dimensions and tissue shrinkage were recorded. The specimens were then subsequently photographed and cut into four equal halves to be processed by conventional, rapid conventional, microwave and automatic tissue processor.

Inclusion criteria:

1. Only excisional biopsied tissues of oral and paraoral region were included in the present study.
2. Only soft tissue specimens were included.
3. Tissue samples of approximately 1cm size or more than 1cm were included in the study.

Exclusion criteria:

1. Incisional biopsies were excluded owing to their small size.
2. Hard tissue samples such as tooth and bone were not included in the study.

Procedure:

Each of the formalin fixed biopsy specimens were cut into four equal halves and subjected to various methods of tissue processing.

The tissues taken for conventional processing were processed as per schedule mentioned below and then embedded in paraffin wax.[2, 5]

Table 1: CONVENTIONAL TISSUE PROCESSING PROTOCOL

STEPS	REAGENT/ PROCESSING FLUID	TIME
Dehydration	70% isopropyl alcohol	45 minutes
	80% isopropyl alcohol	45 minutes
	90% isopropyl alcohol	45 minutes
	100% isopropyl alcohol	1 hour
Clearing	Xylene- 1st change	30 minutes
Clearing	Xylene- 2nd change	30 minutes
Impregnation	Molten paraffin	30 minutes
	Molten paraffin	30 minutes
TOTAL TIME – 315 minutes		

RAPID TISSUE PROCESSING

The tissues taken for rapid processing were processed as per schedule mentioned below and then embedded in paraffin wax.[12]

Table 2: RAPID TISSUE PROCESSING PROTOCOL

STEPS	REAGENT/ PROCESSING FLUID	TIME
Dehydration	70% isopropyl alcohol	30 minutes
	80% isopropyl alcohol	30 minutes
	90% isopropyl alcohol	30 minutes
	100% isopropyl alcohol	30 minutes
Clearing	Xylene- 1st change	20 minutes
Clearing	Xylene- 2nd change	20 minutes
Impregnation	Molten paraffin	15 minutes
	Molten paraffin	15 minutes
TOTAL TIME – 190 minutes		

MICROWAVE TISSUE PROCESSING

Microwave oven commercially available was utilized. Two microwave resistant glass beakers of 200ml each. 100% methyl alcohol as dehydrating agent, 100% isopropyl alcohol as intermedium, paraffin wax, plastic tissue cassettes and hand towel to handle the utensils were used in microwave oven processing of the tissues.

The adequately fixed sample were water washed for 5minutes which removes excess formalin from the tissue. The tissue was transferred to a beaker containing 200ml of 100% methanol for dehydration and the microwave was set at 300W for three 7 minute cycles.

The tissue was transferred into a beaker containing 100% isopropyl alcohol with the same microwave settings. Subsequently the specimens are transferred to a beaker containing 200ml of molten paraffin wax for wax impregnation and the tissue embedding is done in molten paraffin wax immediately. The tissue block thus obtained to be sectioned, dewaxed and then stained with hematoxylin and eosin using microwave oven. The time gap between the irradiation cycles would be almost immediate and as soon as the first cycle is completed, the second cycle would be started.[13, 14]

Table 3: MICROWAVE TISSUE PROCESSING PROTOCOL

STEPS	REAGENT/ PROCESSING FLUID	TIME
Dehydration	100% methyl alcohol	21 min
Intermedium	99% isopropyl alcohol	21 min
Impregnation	Molten paraffin	21 min
	Total time- 63 minutes	

The solutions are not usually covered with the lid because two jars are used; the first jar contains a 200 ml solution (alcohol) along with the tissue inside and the second jar contains a water load of 500ml and is placed next to the first jar. This allows to control the excess heat, which will be absorbed by the water. Subsequently the sections were subjected to routine hematoxylin and eosin staining procedures.

AUTOMATIC TISSUE PROCESSOR

Model: 4S1102, Yorco Co. India, Nominal voltage: 220-240V AC (±10%)

Ergonomic control panel:

The buttons of the control panel were arranged ergonomically for easy handling. The LCD display showed all the parameters throughout the process, such as program number, vessel, remaining time, start time, start delay, total duration of the program, spiral agitation and basket's configuration, temperature of paraffin baths, date and time.

Spiral agitation of the cassette basket during the infiltration process, this function makes it possible for the basket to turn permanently and to automatically change the rotation direction every 60 seconds. This reduces the processing times and obtains a full and homogenous mixture of the reagent plus a perfect infiltration.

Paraffin type:

The four blends of highly refined paraffin and polymers offer a choice of infiltration times and cutting characteristics. Paraffins melt between 55°c-57°c and are triple-filtered to 0.5microns. Turbo flow cassettes are uniquely designed to hold specimens securely and ensure optimal fluid exchange, 85% more than traditional cassettes. This helps to reduce reagent carryover and when used with the Turbo flow base molds, will use less paraffin and virtually eliminate flash. Subsequently the sections were subjected to hematoxylin and eosin staining protocol.[2]

Table 4: AUTOMATIC TISSUE PROCESSING PROTOCOL

Reagent vessel Number	Reagent	Immersion time Hours: Minutes	Stirring time	
			RPM	Programmed value
1	Formalin	01.00	60	1
2	Formalin	01.00	60	1
3	Alcohol 70%	01.30	70	2
4	Alcohol 80%	01.30	70	2
5	Alcohol 96%	01.30	70	2
6	Alcohol 100%	01.00	70	2

7	Alcohol 100%	01.00	70	2
8	Alcohol 100%	01.00	70	2
9	Xylol	01.30	70	2
10	Xylol	01.30	60	1
11	Paraffin	02.00	60	1
12	Paraffin	02.00	60	1
		Total Time- 990 minutes		

HAEMATOXYLIN AND EOSIN STAINING (H & E)

Embedded tissue were sectioned and dewaxed in xylene- two changes of 10minutes each. The tissue sections were hydrated in running tap water for 5minutes. Subsequently slides were transferred to coplin jar containing haematoxylin stain solution for 5-7 minutes and then placed in running tap water for 2-3 minutes for blueing. Sections were differentiated in acid alcohol (one or two dips). Water washing of slide was performed for 5minutes and then transferred to a coplin jar containing eosin stain for 30 seconds. Again water wash of slides is done to remove excess stain and later dehydrated in absolute alcohol for two changes of one dip each. Slides are air dried and mounted.[2]

In the present study, the stained slides processed by conventional, rapid conventional, microwave and automatic tissue processing methods, were subjected to a blind study and each slide was evaluated by four independent observers. The slides were graded into excellent/ good/ average/ poor in a data sheet comprising a total of five parameters. These grading were given numerical value of 4,3,2,1 respectively. And the observers were referred to as 1,2,3,4 respectively.

All the slides were then evaluated by an interobserver analysis for the following criteria:

1. Epithelial tissue
a) Cellular morphology
b) Nuclear morphology
c) Staining characteristics
2. Epithelium and connective tissue interfere
3. Fibrous tissue
a) Cellular morphology
b) Nuclear morphology
c) Staining characteristics
4. Glandular tissue
a) Cellular morphology
b) Nuclear morphology
c) Staining characteristics
5. Muscle tissue
a) Cellular morphology
b) Nuclear morphology
c) Staining characteristics
6. Blood and endothelial cells
a) Cellular morphology
b) Nuclear morphology
c) Staining characteristics
7. Any other tissue of interest

CRITERIA FOR EVALUATION OF QUALITY OF SECTIONS

a) For cellular morphology evaluation; greater eosinophilia of cytoplasm producing enhancement, nuclear cytoplasm contrast, good stroma, whether secretory products are appreciable, absence of red cell lysis, whether differentiation can be made between inflammatory cells were recorded. If most features were present, then it was called as good and if there would be granularity of cytoplasm focal condensation of stroma, cellular outline blurred, mucin not seen, red blood cell lysed(focal or generalized), no differentiation could be made between inflammatory cells then it would be classified as poor.
b) Evaluation of slides for nuclear morphology was done on the basis of chromatin condensation, prominent nuclear membrane and crisp staining of the nucleus and mitotic activity, if appreciable, it was graded as good and poor if smudging and pyknosis of nuclei were observed.
c) Staining of tissues was evaluated as poor, average, good and excellent. Poor indicates that the tissue failed to take up stain adequately, stained unevenly or had artifacts in processing or staining. Average indicates that details were not visualized up to the mark, but slide was suitable to give diagnosis. Good means there is good contrast between the nucleus and cytoplasm, the visibility of details along with brilliance of staining. The overall architecture of epithelial tissue and connective tissue was assessed as per the above mentioned criteria.

SAMPLE SIZE ESTIMATION

Sample size (n) = 40

Sample size (n) = $Z^2{}_{\alpha}PQ/L^2$

Where n = Sample size

Zα = 1.96 for 5 % significant level
P= P- rate 50
Q= 100-50= 50
Permissible error of P
= 30% of P = 15
Sample size (n) = $(1.96)^2$x50x50/ $(15)^2$
= 9604/15x15
= 41.4 (round figure 40)
= 40 sample were selected in study

RESULTS

A total of 40 biopsied tissue specimens were included in the study fulfilling the inclusion and exclusion criteria. The specimens were fixed in 10% formalin for 24 hours to ensure adequate fixation. Each sample was sectioned into four pieces; one of the pieces was sent for conventional manual tissue processing (Type-1), another for rapid conventional tissue processing (Type-2), the third for automatic tissue processing (Type-3) and the fourth for microwave tissue processing (Type-4). The stained slides in each group processed by four techniques were randomly numbered for a blind study and circulated among four observers referred as Observer 1, Observer 2, Observer 3 and Observer 4. The observers graded each parameter by following specific criteria and the observations were tabulated.

Formulation of Hypothesis:
Null Hypothesis: H0 = There is no difference among the different types of tissue processors
Alternate Hypothesis Ha = There is difference among the different types of tissue processors.
P value < 0.05 is considered as statistically significant.
If P value < 0.05 we can reject the null hypothesis and consider the alternate hypothesis.

Table 5: Comparison of observer's opinion of cellular morphology/ nuclear morphology stains in conventional, rapid conventional, automatic and microwave tissue processors

Tissue processors	Quality of stain	Observer 1	Observer 2	Observer 3	Observer 4	Chi-Square test and P-value
Type 1	Good	34(85.0%)	37(92.5%)	32(80.0%)	38(95.0%)	X^2= 3.14
	Poor	6(15.0%)	3(7.5%)	8(20.0%)	2(5.0%)	P> 0.05 NS
Type 2	Good	29(72.5%)	28(70.0%)	30(75.0%)	33(82.5%)	X^2= 2.89
	Poor	11(27.5%)	12(30.0%)	10(25.0%)	7(17.5%)	P> 0.05 NS
Type 3	Good	15(37.5%)	18(45.0%)	21(52.5%)	17(42.5%)	X^2= 1.87
	Poor	25(62.5%)	22(55.0%)	19(47.5%)	23(57.5%)	P> 0.05 NS
Type 4	Good	26(65.0%)	27(67.5%)	24(60.0%)	25(62.5%)	X^2= 1.93
	Poor	14(35.0%)	13(32.0%)	16(40.0%)	19(37.5%)	P> 0.05 NS

(NS: Not Significant)

Inference: The above table shows the comparison of interobserver opinion on cellular and nuclear morphology in stained sections processed by different methods of tissue processing.

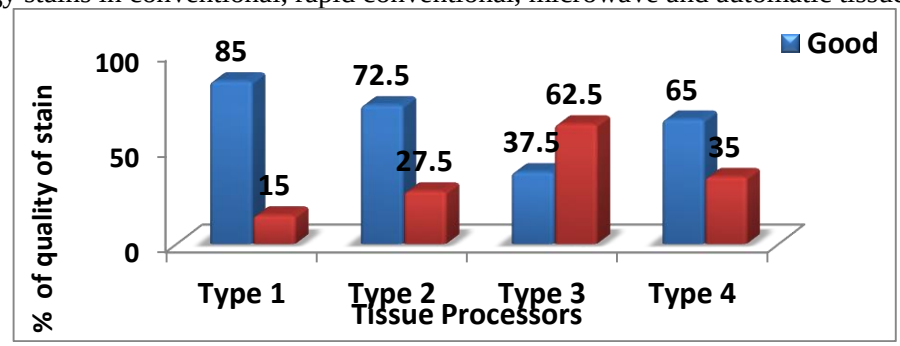
The study observed that, there was no statistical significant difference of opinion on cellular morphology/ nuclear morphology stains in conventional tissue processor among the four observers (P>0.05).

There was no statistical significant difference of opinion of cellular morphology/ nuclear morphology stains in rapid conventional tissue processor among the observers (P>0.05).

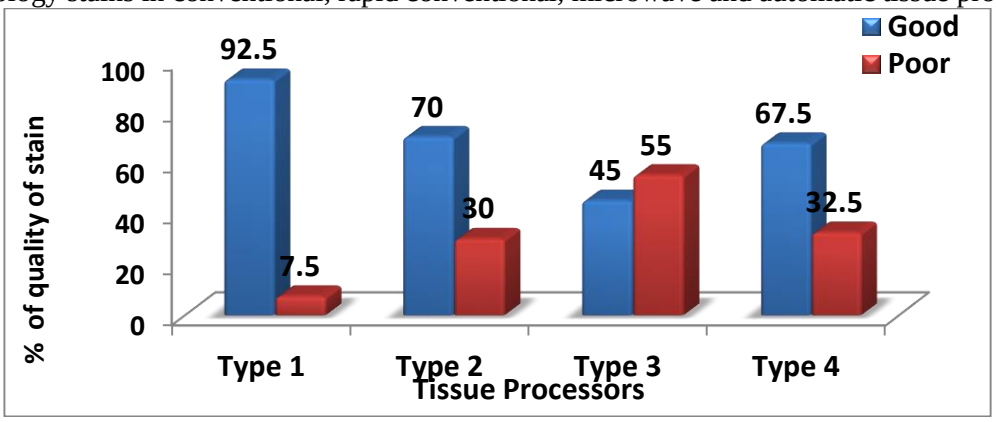
There was no statistical significant difference of opinion of cellular morphology/ nuclear morphology stains in microwave tissue processor among observers (P>0.05)

There was no statistical significant difference of opinion of cellular morphology/ nuclear morphology stains in automatic tissue processor among observers (P>0.05)

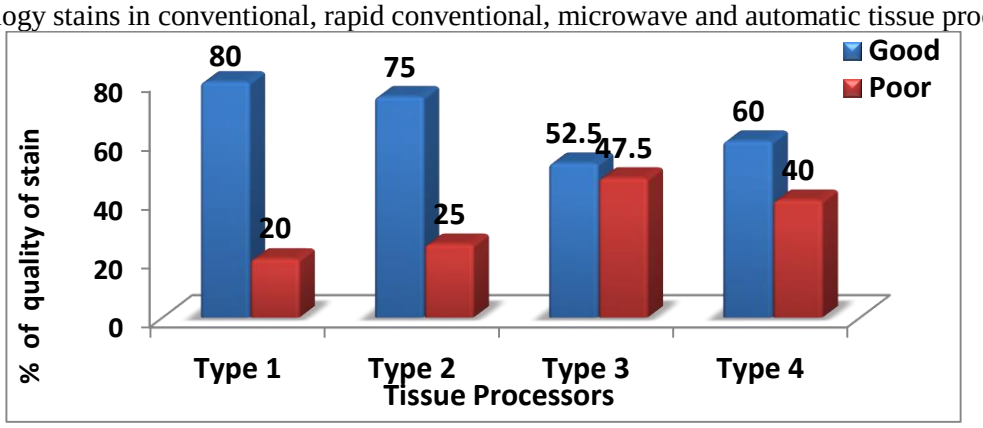
Graph 1: Multiple bar diagram represents opinion of Observer 1 on cellular morphology/ nuclear morphology stains in conventional, rapid conventional, microwave and automatic tissue processors



Graph 2: Multiple bar diagram represents opinion of Observer 2 on cellular morphology / nuclear morphology stains in conventional, rapid conventional, microwave and automatic tissue processors



Graph 3: Multiple bar diagram represents opinion of Observer 3 on cellular morphology/ nuclear morphology stains in conventional, rapid conventional, microwave and automatic tissue processors



Graph 4: Multiple bar diagram represents opinion of Observer 4 on cellular morphology/ nuclear morphology stains in conventional, rapid conventional, microwave and automatic tissue processors

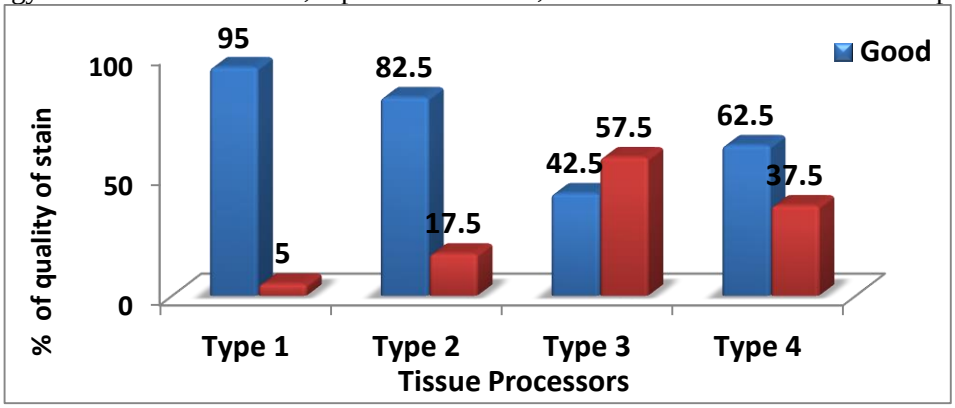


Table 6: Comparison of types of tissue processors of cellular morphology/ nuclear morphology stains

Tissue processors	Type 1		Type 2		Type 3		Type 4		Chi-Square test and P-value
	Good No.(%)	Poor No.(%)	Good No.(%)	Poor No.(%)	Good No.(%)	Poor No.(%)	Good No.(%)	Poor No.(%)	
Observer 1	34 (85.0%)	6 (15.0%)	29 (72.5%)	11 (27.5%)	15 (37.5%)	25 (62.5%)	26 (65.0%)	14 (35.0%)	X^2= 20.16 P< 0.001 HS
Observer 2	37 (92.5%)	3 (7.5%)	28 (70.0%)	12 (30.0%)	18 (45.0%)	22 (55.0%)	27 (67.5%)	13 (32.0%)	X^2= 17.87 P< 0.001 HS
Observer 3	32 (80.0%)	8 (20.0%)	30 (75.0%)	10 (25.0%)	21 (52.5%)	19 (47.5%)	24 (60.0%)	16 (40.0%)	X^2= 15.71 P<0.001 HS
Observer 4	38 (95.0%)	2 (5.0%)	33 (82.5%)	7 (17.5%)	17 (42.5%)	23 (57.5%)	25 (62.5%)	19 (37.5%)	X^2= 18.27 P<0.001 HS

(HS: Highly Significant)

Inference: The above table shows the comparison between the different types of tissue processors based on the cellular morphology/ nuclear morphology of the stained sections. The observations of the study revealed that, there was statistically highly significant difference in cellular morphology/ nuclear morphology stains among types of tissue processor in the first observer (P<0.001).

There was statistically highly significant difference in cellular morphology/ nuclear morphology stains among types of tissue processor in the second observer (P<0.001).

There was statistically highly significant difference in cellular morphology/ nuclear morphology stains among types of tissue processor in the third observer (P<0.001).

There was statistically highly significant difference in cellular morphology/ nuclear morphology stains among types of tissue processor in the fourth observer (P<0.001).

The above data reveals that Type 1 (conventional tissue processor) is significantly better as compared to other types, followed by type 2 (rapid conventional), type 4 (microwave processor) and type 3 (automatic processor).

Table 7: Comparison of observer's opinion of tissue texture stains in conventional, rapid conventional, and microwave and automatic tissue processors

Tissue processors	Quality of stain	Observer 1	Observer 2	Observer 3	Observer 4	Chi-Square test and P-value
Type 1	Good	32(80.0%)	37(92.5%)	34(85.0%)	30(75.0%)	X^2= 3.08 P> 0.05 NS
	Average	5(12.5%)	2(7.5%)	5(12.5%)	7(17.5%)	
	Poor	3(7.5%)	0(0.0%)	1(2.5%)	3(7.5%)	
Type 2	Good	32(80.0%)	28(70.0%)	32(80.0%)	26(65.0%)	X^2= 3.65 P> 0.05 NS
	Average	8(20.0%)	10(25.0%)	5(12.5%)	9(22.5%)	
	Poor	0(0.0%)	2(5.0%)	3(7.5%)	5(12.5%)	
Type 3	Good	15(37.5%)	20(50.0%)	21(52.5%)	17(42.5%)	X^2= 4.67 P> 0.05 NS
	Average	20(50.0%)	8(20.0%)	9 (22.5%)	18(45.0%)	
	Poor	5(12.5%)	12(30.0%)	10(25.0%)	5(12.5%)	
Type 4	Good	33(82.5%)	28(70.0%)	25(62.5%)	23(57.5%)	X^2= 6.41 P> 0.05 NS
	Average	5(12.5%)	6(15.0%)	11(27.5%)	7(17.5%)	
	Poor	2(5.0%)	6(15.5%)	4(40.0%)	10(25.0%)	

Inference: The above table shows the comparison of interobserver opinion on the tissue texture in stained sections processed by different methods of tissue processing.

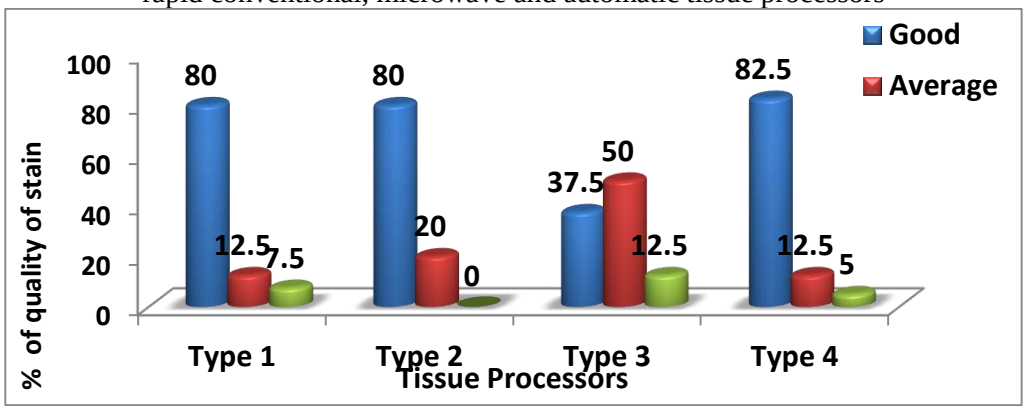
The results reveal that there was no statistical significant difference of opinion of tissue texture stains in the conventional tissue processor among observers (P>0.05).

There was no statistical significant difference of opinion of tissue texture stains in the rapid conventional tissue processor among observers (P>0.05).

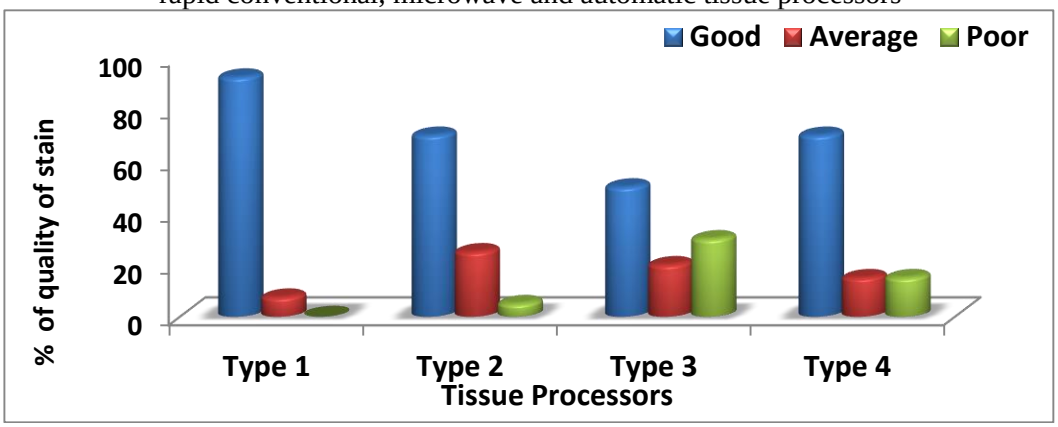
There was no statistical significant difference of opinion of tissue texture stains in the microwave tissue processor among observers (P>0.05).

There was no statistical significant difference of opinion of tissue texture stains in the automatic tissue processor among observers (P>0.05).

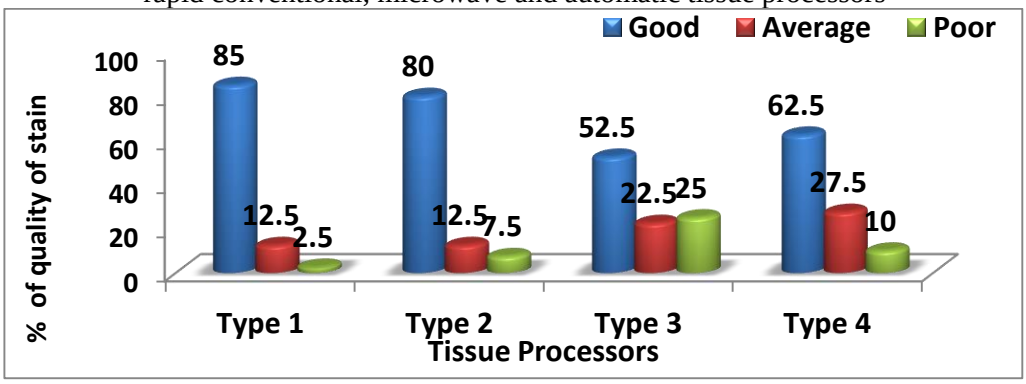
Graph 5: Multiple bar diagram represents opinion of Observer 1 on tissue texture stains in conventional, rapid conventional, microwave and automatic tissue processors



Graph 6: Multiple bar diagram represents opinion of Observer 2 on tissue texture stains in conventional, rapid conventional, microwave and automatic tissue processors



Graph 7: Multiple bar diagram represents opinion of Observer 3 on tissue texture stains in conventional, rapid conventional, microwave and automatic tissue processors



Graph 8: Multiple bar diagram represents opinion of Observer 4 on tissue texture stains in conventional, rapid conventional, microwave and automatic tissue processors

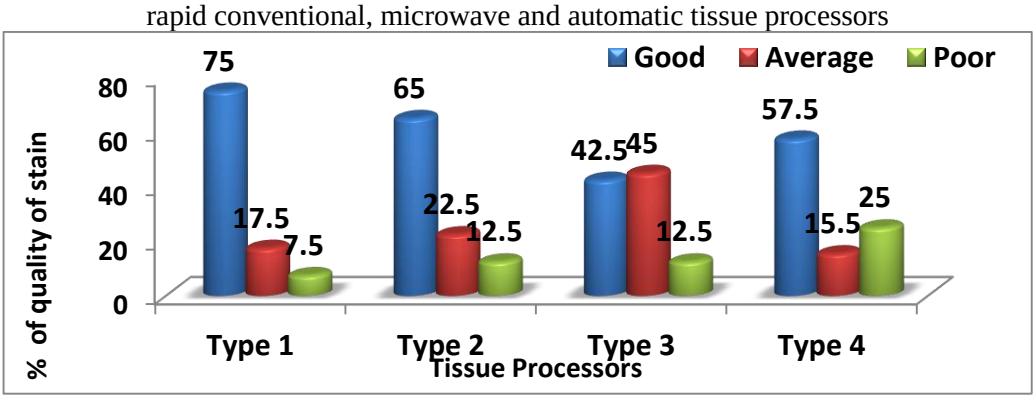


Table 8: Comparison of types of tissue processor in the tissue texture stains

Tissue processors	Quality of stain	Observer 1	Observer 2	Observer 3	Observer 4
Type 1	Good	32(80.0%)	37(92.5%)	34(85.0%)	30(75.0%)
	Average	5(12.5%)	2(7.5%)	5(12.5%)	7(17.5%)
	Poor	3(7.5%)	0(0.0%)	1(2.5%)	3(7.5%)
Type 2	Good	32(80.0%)	28(70.0%)	32(80.0%)	26(65.0%)
	Average	8(20.0%)	10(25.0%)	5(12.5%)	9(22.5%)
	Poor	0(0.0%)	2(5.0%)	3(7.5%)	5(12.5%)
Type 3	Good	15(37.5%)	20(50.0%)	21(52.5%)	17(42.5%)
	Average	20(50.0%)	8(20.0%)	9 (22.5%)	18(45.0%)
	Poor	5(12.5%)	12(30.0%)	10(25.0%)	5(12.5%)
Type 4	Good	31(77.5%)	28(70.0%)	25(62.5%)	23(57.5%)
	Average	7(17.5%)	6(15.0%)	11(27.5%)	7(17.5%)
	Poor	2(5.0%)	6(15.5%)	4(40.0%)	10(25.0%)
Chi-Square test and P-value	-----	X^2= 24.32 P<0.001 HS	X^2= 20.72 P<0.001 HS	X^2= 19.58 P<0.001 HS	X^2= 22.32 P<0.001 HS

Inference: The above table shows the comparison between the different types of tissue processors based on the tissue texture of the stained sections. The study revealed that, there was statistically highly significant difference in texture stains in the types of tissue processor among the first, second, third and fourth observers (P<0.001). Type 1 (conventional tissue processor) is significantly better as compare to other types, followed by type 2, type 4 and type 3.

Table 5: Comparison of types of tissue processors based on cellular morphology/ nuclear morphology stains with total time

Types	Type 1		Type 2		Type 3		Type 4	
Total Time	315 minutes		190 minutes		990 minutes		63 minutes	
Observers	Good No.(%)	Poor No.(%)	Good No.(%)	Poor No.(%)	Good No.(%)	Poor No.(%)	Good No.(%)	Poor No.(%)
Observer 1	34 (85.0%)	6 (15.0%)	29 (72.5%)	11 (27.5%)	15 (37.5%)	25 (62.5%)	26 (65.0%)	14 (35.0%)
Observer 2	37 (92.5%)	3 (7.5%)	28 (70.0%)	12 (30.0%)	18 (45.0%)	22 (55.0%)	27 (67.5%)	13 (32.0%)
Observer 3	32 (80.0%)	8 (20.0%)	30 (75.0%)	10 (25.0%)	21 (52.5%)	19 (47.5%)	24 (60.0%)	16 (40.0%)
Observer 4	38 (95.0%)	2 (5.0%)	33 (82.5%)	7 (17.5%)	17 (42.5%)	23 (57.5%)	25 (62.5%)	19 (37.5%)
Average Percentage	88.13%	11.87%	75.0%	25.0%	44.37%	55.63%	63.75%	36.25%

Inference: The above table shows the comparison between different types of tissue processors based on cellular morphology/ nuclear morphology of the stained sections with the total time of processing. The study observed that, type 1 (conventional tissue processor) method had 88.13% of good stain with 315 minute total time followed by type 2 (rapid conventional tissue processor) method had 75.0% of good stain with 109 minutes of total time and next better method was type 4 (microwave tissue processor) had 63.75% of good stain with total time was 63 minutes and next poor method as compare to other methods (automatic tissue processor) had 44.37% of good stain with 990 minutes of total time.

Table 6: Comparison of types of tissue processor in the texture stains with total time

Tissue processors	Quality of stain	Observer 1	Observer 2	Observer 3	Observer 4	Average Percentage
Type 1 315 minutes	Good	32(80.0%)	37(92.5%)	34(85.0%)	30(75.0%)	83.12%
	Average	5(12.5%)	3(7.5%)	5(12.5%)	7(17.5%)	12.50%
	Poor	3(7.5%)	0(0.0%)	1(2.5%)	3(7.5%)	4.38%
Type 2 190 minutes	Good	32(80.0%)	28(70.0%)	32(80.0%)	26(65.0%)	73.75%
	Average	8(20.0%)	10(25.0%)	5(12.5%)	9(22.5%)	20.0%
	Poor	0(0.0%)	2(5.0%)	3(7.5%)	5(12.5%)	6.25%
Type 3 990 minutes	Good	15(37.5%)	20(50.0%)	21(52.5%)	17(42.5%)	45.63%
	Average	20(50.0%)	8(20.0%)	9 (22.5%)	18(45.0%)	34.37%
	Poor	5(12.5%)	12(30.0%)	10(25.0%)	5(12.5%)	20.0%
Type 4 63minutes	Good	31(77.5%)	28(70.0%)	25(62.5%)	23(57.5%)	66.87%
	Average	7(17.5%)	6(15.0%)	11(27.5%)	7(17.5%)	19.37%
	Poor	2(5.0%)	6(15.5%)	4(40.0%)	10(25.0%)	13.76%

Inference: The above table shows the comparison between the different types of tissue processors based on the tissue texture of the stained sections with the total time of processing. The results revealed that, type 1 (conventional tissue processor) method had 83.12% of good stain and 12.5% of average stain with 315 minute total time followed by type 2 (rapid conventional tissue processor) method had 73.75% of good stain and 20.0% had average stain with 190 minutes of total time and next better method was type 4 (microwave tissue processor) had 66.87% of good stain and 19.37 of average stain with total time was 63 minutes and next poor method as compare to other methods (automatic tissue processor) had 45.63% of good stain and 19.37% of average stain with 990 minutes of total time.

Figure 1: Photomicrograph showing section obtained after conventional tissue processing method

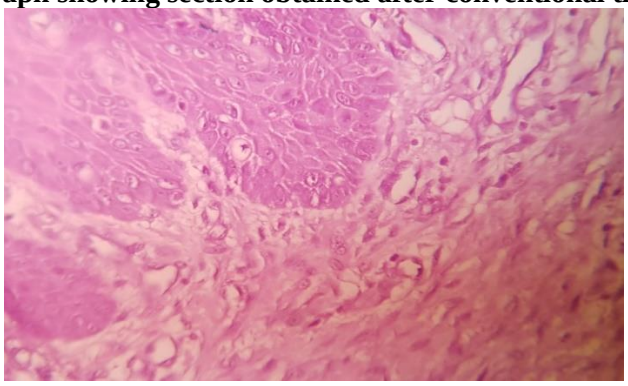


Figure 2: Photomicrograph showing section obtained after rapid conventional tissue processing method

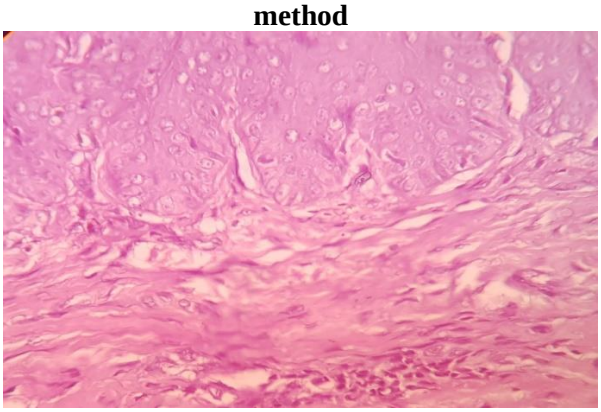


Figure 3: Photomicrograph showing section obtained after microwave tissue processing method

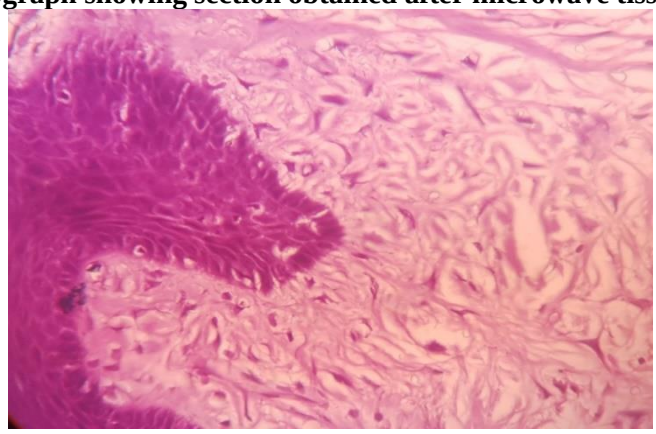
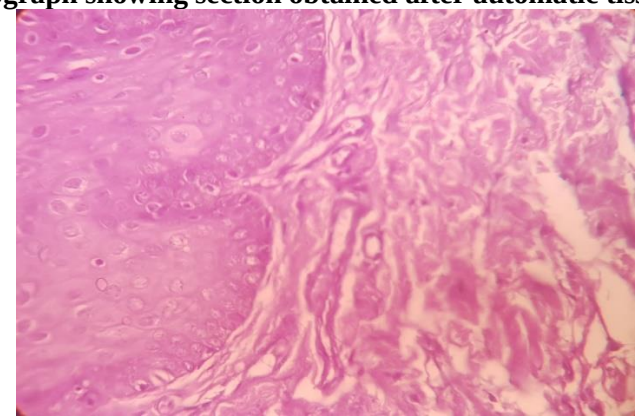


Figure 4: Photomicrograph showing section obtained after automatic tissue processing method



DISCUSSION

Processing techniques that give rise to adequate tissue quality were established for most animal tissues many years ago. Many of these protocols take days to complete, for numerous application and the lengthy duration required for processing tissues in microscopy are an impediment.[15] This is true, for example, in clinical samples in which microscopic examination or diagnosis are based on or at least in part - on microscopic structural findings and for research studies in which procedures or protocol modification must await microscopic structural results.[16]

Tissue processing is an important histotechnique after biopsy procedure. It involves passing of the biopsy tissue in graded concentration of various chemicals in order to make the tissue amiable for sectioning. This includes dehydration, clearing and infiltration.

Tissue contains water molecules and will not allow the embedding media to enter. The process of dehydration is needed to replace the water in the tissue by alcohol or a substitute; clearing comprises of the exchange of alcohol by a reagent miscible with paraffin or its substitute; and impregnating is the process in which the clearing agent is replaced by paraffin or its substitute. The physicochemical basis of tissue processing lies in the diffusion of reagents into the substance of the tissue to be processed. The diffusion process results from the thermodynamic tendency of processing reagents to equalize concentrations inside and outside blocks of tissue.[17]

The practice of histopathology during the last five decades has been enriched by advances in our knowledge of morphologic expression of disease and by new technologies such as immunohistochemical and molecular assays. Handling of tissue samples from surgical removal to the preparation of H and E stained slides, however, has remained impervious to scientific advances.[11]

Diagnostic pathology is largely dependent on formalin-fixed paraffin-embedded tissue sections. In particular, formalin fixation, followed by currently used conventional processing methods has been the standard for almost 100 years.[18] A 1-day minimum delay in the preparation of diagnostic cases, toxicity of reagents used, and degradation of nucleic acids, are a few of the important shortcomings associated with that practice.

Rapid manual tissue processing is of a shorter duration than the conventional method, requiring 3-4h. It includes the same steps as in routine method, but for shorter durations. The advantage is that it consumes only 20% of time as compared to routine method.[19]

Automation in tissue processing may hasten the process by continuing beyond the working hours of the laboratory, but it needs continuous power supply which cannot be ensured all the time. Automation is also an expensive affair. The high cost of automation and unavailability of uninterrupted power supply in the developing countries makes automation unsuitable for small laboratories. The excessively long cycle chosen for the automatic processor leads to poor quality of sections.[20]

The current research compared the four different methods of tissue processing- conventional, rapid conventional, automatic and microwave processing and also assessed its impact on the staining quality of the sections. The quality of the histologic preparation was compared by four pathologists who were to assess whether the slides were good, average or poor. This was done in a blinded fashion. The statistical analysis revealed that Type 1 (conventional tissue processor) is significantly better as compared to other types, followed by type 2 (rapid conventional), type 4 (microwave processor) and type 3 (automatic processor).

The present study also compared the interobserver opinion on the cytoplasmic and nuclear details in the hematoxylin and eosin stained tissue sections processed by conventional, rapid conventional, automatic and microwave techniques. The study observed that, there was no statistical significant difference of opinion on cellular and nuclear morphology of the sections in all the types of tissue processors among the four observers ($P>0.05$). The effect of the four methods of tissue processing on cytoplasmic and nuclear details as assessed in terms of epithelial and connective tissue cells, showed no statistically significant variation. Our observations were in accordance with the findings of Panja P et al[19] and Chaudhari et al[21], who also demonstrated that the quality of staining was similar in the different processing techniques. Boon et al found that in microwave processed tissues the epithelium was of better quality and the stroma showed more focal condensation.[7]

In our study, we compared the different types of tissue processors based on the quality of the tissue texture of the stained sections and we found that there was a statistically highly significant difference in the quality of tissue texture stains processed in the different types of tissue processors among the first, second, third and fourth observers ($P<0.001$). The quantitative analysis revealed that Type 1or conventional tissue processing is significantly better ($X^2= 24.32$) as compared to other types, followed by type 2 or rapid conventional processing ($X^2= 20.72$) then type 4 or microwave processing ($X^2= 22.32$) and type 3 or automatic processing ($X^2= 19.58$).

The results of the interobserver opinion found the tissue texture and the stroma of the hematoxylin and eosin stained sections to be of the same quality between the tissues processed by conventional, rapid conventional, automatic and microwave tissue processors; except for microwave processed tissue which showed brighter staining with eosin compared to other processed sections. Morales et al also observed similar findings in their study.[8]

Our study demonstrated no substantial differences in overall quality or diagnosability of tissue sections prepared by the different processing techniques. Our observations are in consensus with the findings of Leong et al and Mathai AM et al, who described the quality of sections obtained from different tissue processing methods as indistinguishable.[9,22]

During the last 30 years, microwave-assisted tissue processing has been studied. The technique has achieved increasing acceptance in the last decade. The increased popularity of microwave-assisted tissue processing has led to the production of commercially available microwave ovens specifically designed to ensure uniform rapid tissue processing under precisely controlled specimen temperatures. These machines also precisely control the on-off cycling of the heating. Such commercial units have facilitated accomplishment of tissue processing and diagnosis on the same day in which the specimen was obtained.[23]

Microwave processing changes the procedure considerably; it permits a more rapid completion of fixation before the initiation of histologic processing. Dehydration is achieved in 1 step instead of multiple graded solutions of alcohol, and paraffin impregnation occurs at a higher temperature, speeding the process. In principle,

microwave processing uses previously fixed tissue that is dehydrated rapidly using microwave energy to heat the reagent alcohol to just below its boiling point. Isopropanol further dehydrates the tissue and prepares it for paraffin infiltration. The residual isopropanol is effectively boiled out by using microwave energy to heat liquid paraffin above the boiling point of isopropanol. This procedure substantially shortens the processing time without degradation of histomorphologic features. Use of microwave methods considerably reduces the processing time with 1-step dehydration and 1-step clearing before paraffin infiltration.[24]

Microwave irradiation has several advantages over routine conventional methods from the perspective of laboratory personnel. It also has certain environmental advantages. It eliminates the need for xylene in tissue processing. From the perspective of the final product, microwave irradiation substantially shortens the time from specimen reception to diagnosis.[22, 25]

In the present study the microwave method showed that the stroma has a slightly different appearance in that it seems to be a bit more condensed focally. The epithelium in the microwave sections is often of better appearance. The staining characteristics are identical. In almost all cases, there was no qualitative difference between the techniques.

The disadvantage of microwave method is that the machine is expensive. The tissue section must be no more than one cubic centimeter when fixed, otherwise complete and even penetration of microwaves will not result. Microwaves can penetrate to a maximum depth of 2 cm and hence they may require a few additional cycles of dehydration in larger tissues.[22]

In our study we compared the different types of tissue processors based on the quality of staining with the total time required for processing, the results revealed that, type 1 (conventional tissue processor) method had 83.12% of good stain and 12.5% of average stain with 315 minute total time followed by type 2 (rapid conventional tissue processor) method had 73.75% of good stain and 20.0% had average stain with 190 minutes of total time and next better method was type 4 (microwave tissue processor) had 66.87% of good stain and 19.37% of average stain with total time was 63 minutes and next poor method as compare to other methods (automatic tissue processor) had 45.63% of good stain and 19.37% of average stain with 990 minutes of total time.

We believe that rapid microwave-assisted tissue processing is the optimal method for substantially reducing turnaround time and permitting the histopathology laboratory to consistently provide same-day diagnosis for a variety of types of tissue biopsy specimens.

Rapid processing of histopathologic material is becoming increasingly desirable for intraoperative consultations and timely diagnosis.[14] We found positive impact on turnaround time in microwave method as the time taken for block preparation from fixed tissue was 1hour as compared to conventional method which was 315 minutes, rapid manual method was 190 minutes and automatic processing method was 990 minutes.
According to our study we found that the conventional tissue processing method is significantly better when compared to other methods of tissue processing. The conventional tissue processing is reliable and cost effective. We continue to maintain conventional tissue processing method as the gold standard for histologic examination.

CONCLUSION

Tissue processing as a method in which tissues are preserved by manual or automatic means to retain their life like state and cellular components for further analysis by pathologists. Tissue processing forms the basis of tissue diagnostics, which is a fundamental tool in the study of pathological diseases. The ability to obtain good quality of tissue sections would be valuable for diagnostic pathology. The study here concludes that, good quality of tissue sections could be obtained by conventional tissue processing technique. The histological assessment of the hematoxylin and eosin stained sections obtained by the conventional tissue processing method were comparatively better than those obtained by other methods. This was followed by rapid conventional method, then microwave method and automatic processing method.

***Briefly, this study concluded that*;**

1. conventional tissue processing method is a reliable method
2. microwave tissue processing considerably shortens the turnaround time
3. there are no substantial differences in overall quality or diagnosability of tissue sections prepared by the different processing methods.

Patients rely on quality tissue processing and most laboratory supervisors would emphasize to their staff the importance of tissue processing. It is worthwhile to stress that the use of an inappropriate processing schedule or the making of a fundamental mistake (perhaps in replenishing or sequencing of processing reagents) can result in the production of tissue specimens that cannot be sectioned and therefore will not provide any useful microscopic information.

Microwave tissue processing method allows same day tissue processing and diagnosis of small biopsy specimens without compromising the overall quality of the histologic section, thus improving the workflow of the laboratory.
The overall morphology, overall staining, cellular outline, cytoplasmic and nuclear details of tissues processed by conventional method were comparable to or superior to that processed by the other methods.
Finally, it can be recommended that the conventional tissue processing method is the gold standard for histologic examination.

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