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Original Article

Microwave histoprocessing versus conventional histoprocessing

Alka Mary Mathai, Ramadas Naik, Muktha R. Pai, Sharada Rai, Poornima Baliga

Department of Pathology, Kasturba Medical College, Mangalore, Karnataka, India

Address for correspondence:
Dr. Ramadas Naik, Department of Pathology, Kasturba Medical College, Mangalore - 575 001, Karnataka, India.
E-mail: drrdn@hotmail.com

ABSTRACT

The aim of the study is to compare the histologic quality of the microwave histoprocessing with that of conventional method and to determine its positive impact on turnaround times and reduction of costs of tissue processing. One hundred and eighty-five paired tissue sections from different organs were taken. Each tissue sections were of size of 15 mm × 10 mm × 3 mm and divided into two; one set as experimental group and the other as control group. The tissues in the experimental group were further divided into six groups and processed by vacuum-microwave method according to six protocols from I to VI. Other tissues in the control group were processed by the conventional method and compared. Overall, the quality of microscopic tissue from both the methods was identical. Microwave processing shortened the time of processing without compromising the overall quality of the histologic section and was cost-effective.

KEY WORDS: Microwave, histoprocessing, histologic quality, turnaround time

INTRODUCTION

The applications of microwaves in histopathology has gained acceptance in the last two to three decades. It has been used for virtually all procedures in pathology: stabilizing tissues, fixation, histoprocessing, staining, frozen techniques, immunotechniques, techniques for electron microscopy and antigen retrieval.[1] In the same decade, vacuum in combination with the microwave processor was introduced.

Microwave causes heating within a material by exciting molecules to rotate. The rotation produces energy in the form of heat. Heat reduces the viscosity of liquids, thereby increasing the rate of diffusion of reagents into and out of the tissue. Unlike conventional heating, the effect occurs simultaneously throughout the whole material being microwaved ('internal heating').[2] This resulted in substantial reduction in each of the basic steps of histoprocessing, thereby reducing turnaround times and permitting same day diagnosis for a variety of types of tissue biopsy specimens. The aim of the present study is to document the usefulness of microwave-assisted tissue processing, to determine whether it can replace the standard conventional processing, to compare the histologic quality with that of the conventional method and to determine its positive impact on turnaround times and reduction of costs of tissue processing.

MATERIALS AND METHODS

The present study was conducted in the Department of Pathology, Kasturba Medical College, Mangalore. The duration of the study was from July 2003 to June 2005. Specimens for the study were selected randomly from those received in the department from associated hospitals of Kasturba Medical College, Mangalore and private hospitals and clinics in and around Mangalore.

One hundred and eighty-five paired tissue sections from different organs including gastrointestinal tract, thyroid, ovary, endometrium, cervix, gall bladder, salivary gland, lymph node and soft tissues were taken [Table 1]. Specimens with both neoplastic and non-neoplastic lesions were studied. All tissue sections were of standard size of 15 mm × 10 mm × 3 mm. Each section was divided into two. One set of sections was labeled as experimental group and the other set as control group. The tissues in the experimental group were further divided into six groups and processed by vacuum-microwave method according to six protocols from I to VI, other tissues in the control group being processed by the conventional method [Table 2]. The microwave system used was the Milestone RHS-I vacuum histoprocessor, which has all programs fully loaded to run at appropriate times and temperatures.

Fixation in microwave was done in the microwave unit with 10% buffered formalin for 20 min at 50°C. Dehydration of the tissue blocks was done with methanol. The cassettes in methanol were simultaneously exposed to microwave and to vacuum. The intensity of microwave exposure was regulated by temperature control. A temperature of 35-40°C in this step was programmed for 20 min. After dehydration, the cassettes were placed in isopropyl alcohol and again exposed to microwaves and vacuum. A temperature of 70°C was programmed at a pressure of 700 hPa for 120 min. At these settings, no adverse effects were produced to the final morphology of the tissues and at the same time clearing was complete. The next paraffin step took 20 min. The pressure

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was brought down gently from 700 hPa to a lower value of 100 hPa, during which, the temperature of paraffin was above the boiling point of water, methanol, and isopropyl alcohol. Therefore, by lowering the pressure and the boiling point, the reagents within the tissues could be easily 'boiled out' for perfect impregnation by wax. To ensure adequate removal of isopropyl alcohol, a vacuum drying step was introduced, where pressure was brought down from 700 to 500 hPa. The traces of water left in the tissues were also removed. A separate container was used for the paraffin step to avoid contamination of the dehydration container with paraffin. In all the three steps, the volume of the reagents was such that all the cassettes were completely immersed.

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A retrospective review of turnaround times for tissue processing from specimen receipt to completion of haematoxylin- and eosin-stained sections by microwave technique was performed and compared with similar turnaround times for specimens processed conventionally prior to introduction of microwave processing.

Table 1: The types of tissues taken in the study

Type of tissue	*Number of cases*	*Percentage*
Gastrointestinal tract	40	21.61
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A non-parametric Mann-Whitney *U*-test was used for statistical analysis of differences between the control and the experimental group. *p*-values less than 0.05 were considered significant.[3,4]

RESULTS

Protocols III and V gave the best scores of overall morphology and Protocol I gave the lowest scores. Overall staining of Protocol V was the best. Protocol III gave the best scores in cellular outline and Protocol VI gave the lowest scores. Cytoplasm details were the best in Protocols III and IV. Protocols III and V gave the best nuclear detail scores and Protocol I gave the lowest scores. Protocols II, III, IV and V gave the best scores in erythrocyte integrity and lymphocyte appearance [Figure 1]. Overall histologic quality was better in Protocol III ($p = 0.022$ sig), where wax impregnation alone was done in microwave as compared to the controls [Table 3].

In protocol I, tissue sections from gastrointestinal tract and thyroid showed significant difference in the fixation ($P = 0.05$ sig and $P = 0.008$ hs, respectively), where microwave fixation was better. In all the other protocols, there was no significant difference in the processing when the type of organ was taken into account.

When the type of lesion was taken into account, malignant lesions showed significant difference in the processing in Protocol II ($p = 0.015$ sig) and Protocol III ($P = 0.026$ sig), where microwave processing was better [Figure 2]. The tissue sections had better cytoplasmic and nuclear details with good erythrocyte integrity and lymphocyte appearance than the conventional method. For other tissues of normal histology, non-neoplastic and benign lesions, there was no significant difference in the processing in

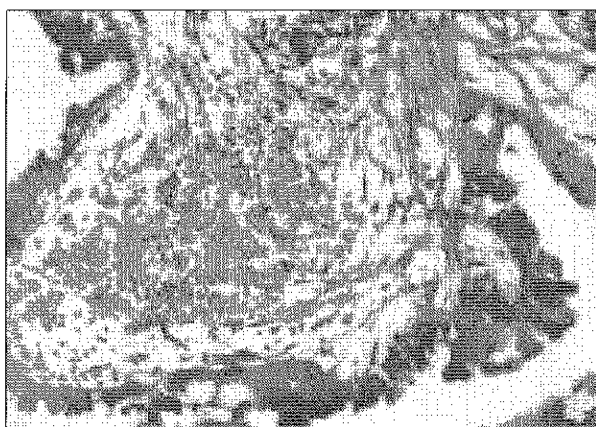

Figure 1: Excellent erythrocyte integrity - Protocol IV (H&E, x400)

Table 2: The seven protocols used in the study

Steps	*P I*	*P II*	*P III*	*P IV*	*P V*	*P VI*	*P VII*
Fixation	MW	C	C	MW	C	MW	C
Dehydration	C	MW	C	MW	MW	MW	C
Clearing	C	MW	C	MW	MW	MW	C
Wax impregnation	C	C	MW	C	MW	MW	C
Duration	15 hrs 20 min	18 hrs 20 min	7 hrs 20 min	14 hrs 40 min	6 hrs 40 min	3 hrs	19 hrs

P = Protocol, MW = Microwave, C = Conventional

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Table 3: Comparison between microwave and conventional methods in all the protocols

Protocol	*Method*	*N*	*Mean*	*Std. Deviation*	*Z*
I	Microwave	31	20.7097	2.35504	1.272,
	Conventional	31	19.8387	2.84132	p = 0.185 ns
II	Microwave	31	21.3226	1.24866	1.876,
	Conventional	31	19.9677	4.26211	p = 0.065 ns
III	Microwave	31	21.4194	0.84751	2.482,
	Conventional	31	20.7742	1.14629	p = 0.022sig
IV	Microwave	31	21.4516	1.05952	1.769,
	Conventional	31	20.9677	1.27760	p = 0.413 ns
V	Microwave	31	21.2903	0.90161	0.394,
	Conventional	31	21.3548	0.98483	p = 0.694 ns
VI	Microwave	30	20.6333	2.28161	0.405,
	Conventional	30	20.4000	1.86806	p = 0.672 ns

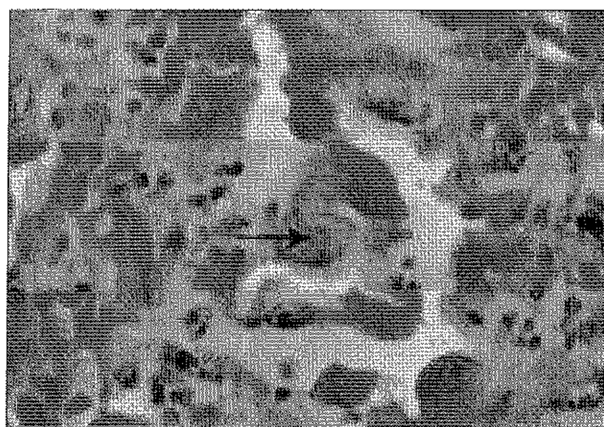

Figure 2: Prominent nucleolus (arrow) in melanoma cells- Protocol II (H&E, ×1000)

any of the protocols. The endoscopic biopsies processed along with the large tissues also produced excellent results with the same temperature control.

The microwave technique did not produce any deleterious effects on special stains. Periodic acid - Schiff, van Gieson, Congo red, Masson's trichrome, alcian blue, mucicarmine and reticulin staining pattern and intensities on tissue processed by microwave method could not be distinguished from those by conventional method.

Decalcification of bone with 5% formic acid using vacuum-microwave histoprocessor was studied. The final sections were excellent when compared with conventional processing with a drastic reduction in time from days to a maximum of 9 h.

DISCUSSION

A potential application of microwave energy in histotechnology was first recognized by Mayers in 1970.[5] This form of non-ionizing radiation produces alternating electromagnetic fields that result in the rotation of dipolar molecules such as water and the polar side chains of proteins through 180° at the rate of 2.45 billion cycles/second. The molecular kinetics so induced results in the generation of energy flux and continues until radiation ceases.

Boon *et al.* reported that it is possible to produce significant acceleration of tissue processing by introducing microwaves to the procedure.[2] Kok and Boon described a novel technique, where vacuum was combined with microwaving.[6] They have exploited extensively the fact that the boiling temperature of liquids featured in histoprocessing decreases when the liquid is placed in vacuum. Accordingly, vacuum was used to 'pull out' the molecules. Moreover, this can be done at relatively low temperatures, so that the tissue is protected against the negative effects of overheating. A similar vacuum-microwave histoprocessing technique was used in the present study.

A specially designed Milestone RHS-1 vacuum histoprocessor was used. The microwave unit had width of 20', depth of 27.3' and height of 22.8', with maximum power of 800 W, 230 V. The vacuum chamber had an infra-red sensor, a fully automated non-contact temperature control, an exhaust fan to extract vapors so that no toxic vapors are released to laboratory, a vacuum outlet to improve extraction of solvents from tissue and for wax impregnation and an auto start magnetic stirrer for homogenous temperature distribution within the solution eliminating 'hot spots'. For vacuum-microwave steps, an external vacuum module condenser was developed to collect the vapors from the RHS unit before they went into the vacuum module controller. The instrument was equipped with software regulating power output, temperature and time. The plastic cassettes, each one containing one tissue block, were put in one specially designed holder. The holder used could contain a maximum of 110 cassettes. The loaded holder was put in a glass container of 5 l capacity and kept in the vacuum chamber. The thickness of each of the tissue sections was of the order of 0.5 cm similar to that in previous studies,[7-9] so that the microwave excitation was virtually uniform throughout the tissue.

After each step, the volume of the tissue had decreased. The decrease in volume varies between different tissues and is similar to that of the conventional technique. However, the microscopical results of both the methods were excellent in concordance with that of Kok *et al.*[8] Microwave processing was less effective in processing large number of tissues. Therefore, the reagents had to be changed every third day when 110 tissue sections were processed at a time. Similar problem was encountered by Kok *et al.*[8] The microwave oven did not produce any 'hot spots' or the uneven heating encountered in smaller domestic microwave oven; mainly because of the magnetic stirrer kept beneath the rack, which provides an even field of irradiation. The magnetic stirrer is very important in the third step of wax impregnation. The third step should be carried out immediately, otherwise the molten paraffin gets solidified and the stirrer does not rotate, resulting in increase in pressure in the chamber, stopping the program. This was overcome by keeping the microwavable container with molten paraffin permanently in the hot air oven.

The present study compared the histologic quality of the slides processed by microwave method and the conventional method. The parameters and the ratings of the adequacy used were similar

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to those of Titford and Horenstein.[10] Fixation in microwave with 10% buffered formalin for 20 min at 50°C was compared with the conventional method. The findings were in agreement with those of Gordon.[11] There was no erythrocytolysis, indicating good performance in the fixation of cellular membrane as observed by Titford and Horenstein and Boon *et al.* There was no significant difference in the nuclear size and the chromatin pattern was reticular with good lymphocyte appearance scores, indicating good performance in the fixation of chromatin. These findings were similar to those of Titford and Horenstein. Excellent cytological preservation with good preservation of nuclear membranes, adequately clumped chromatin and well-defined cellular outlines noted in the present study were in agreement with those of Gordon.[11] Among the various organs processed, there was significant difference in the fixation of gastrointestinal and thyroid tissues, where the microwave method was superior ($P = 0.05$ sig and $P = 0.008$ hs, respectively). Other organs did not show any significant difference. In all the sections, the tissue architecture was well-maintained with no shrinking or spongy pattern. No crisp ethyl alcohol patterns of nuclear features were seen as observed by Boon *et al.* [8] No microwave-induced vulnerability such as condensation and shrinkage of surface tissue with nuclear pyknosis, loss of cytoplasmic details and lakes of red blood cells in the region of shrinkages was noted in contrast to those observed by Gordon who exposed tissues to microwaves in air. Therefore, there was uniform formaldehyde fixation. It was significant that microwave fixation followed by routine conventional method did not alter the tissue morphology and cellular details or the criteria for establishing malignancy. The major advantage of microwave fixation was the marked reduction in the time required for the fixation of tissue prior to dehydration and hardening, taking approximately 20 min when compared to hours in conventional method. Moreover, the microwave fixed tissues were more easily cut on the microtome as claimed by Bezahler.[12]

Dehydration and clearing were done in microwave at 70°C for 2 h. Macroscopically, the tissue blocks did not shrink during histoprocessing and paraffin was observed to have impregnated everywhere in the tissue. The paraffin blocks were easy to cut. Malignant lesions showed better histologic quality with microwave dehydration and clearing ($P = 0.015$ sig).

Wax impregnation alone was done in the microwave unit for 20 min for 31 tissues and compared with the conventional method. There was significant difference in the overall staining when compared with the controls ($P = 0.049$ sig), where 28 (90.3%) sections had uniform good staining. This may be attributable to the vacuum step introduced prior to wax impregnation, where the residual isopropyl alcohol in between the tissues is removed, permitting uniform wax impregnation. Irregular nuclear membrane, prominent nucleoli and mitotic figures in malignant lesions were also clearly evident in the tissue sections. Malignant lesions showed significantly better histologic features when wax impregnation was done in microwave method ($P = 0.026$ sig). Microscopically similar features were observed when dehydration, clearing and wax impregnation were combined in the microwave method. Overall, the quality of microscopic tissues from conventional processing and microwave processing methods were identical. It was not possible to distinguish between the two techniques by studying the tissue section.

In Protocol VI, all the steps were combined in microwave simulated method. Noteworthy was the complete absence of tissue shrinkage during histoprocessing. This was highly important for quantitative pathology, where objective measures were required, for example, for depth of invasion or volume of tumor. There was no significant difference between nuclear size and shapes, and staining characteristics were discernable. Similar findings were observed by Sivadas *et al.*[13] where alcohol, chloroform and wax were used for processing. However, cytoplasm stained moderately eosinophilic with no deeper eosinophilia as observed by them.

For routine purposes, it is often desirable to obtain the paraffin sections in a few hours, but this is not possible with the conventional method. In the present study, the microwave processing with Protocol VI met the above demand. Protocol VI, where all the steps of fixation, dehydration, clearing and wax impregnation were done in microwave, took a total processing time of 3 h. Thus, in this manner, it was possible to run several short cycles of about 3 h each during the working day so that stained sections were available on the same day as the specimens were received. Endoscopic biopsies were processed through a short cycle of 40 min using the same reagents. In our routine practice, we processed biopsies and resected specimens together, as such sampling error is not a problem with vacuum-microwave processing, which was noted by Suri *et al.*[14] But despite the advantages, microwave procedure is still not practiced widely.[15] This is mainly due to the established working schedule in the laboratories. With conventional processing, the workload is maximum in the morning, while microwave processing produces continuous even activity during the day, which is not easily accepted by laboratory staff. Therefore, in the routine setup of this laboratory, Protocol IV of microwave processing was found suitable, taking into consideration the working pattern of technical staff who worked daily from 9:00 am to 5:00 pm. All the specimens that reached the laboratory till 4:00 pm were grossed and fixation, dehydration and clearing were done in microwave on the same day. One of the laboratory officials had to stay after 5.00 pm to keep the tissues in molten paraffin wax where it remained overnight. The next day the sections were embedded and slides were made available to the pathologists for reporting by 2:00 pm. Even though the whole procedure takes 14:40 hours, the slides were ready for reporting next day afternoon. This was 1 day ahead when compared with conventional processing where the duration of processing was 19:00 hours and the slides for reporting were made available after 2 days.

Microwave processing had several advantages over routine method. It eliminates the use of xylene from the processing and also formalin as determined by the laboratory. Microwave processing substantially shortens the time from specimen reception to diagnosis without compromising the overall quality of the histologic section.

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Since isopropyl alcohol was also a dehydrating agent, processing with isopropyl alcohol and wax, a two-step procedure was done in microwave processor as suggested by Boon *et al.*[2] This method could save ethanol or is useful when ethanol is not available. However, the method described in the present study, a three-step procedure with methanol, isopropyl alcohol and paraffin combined with vacuum gave excellent results than the two-step procedure. Use of reagents was minimal in this method and the paraffin can be used several times. In the present study, the used isopropyl alcohol was discarded every third day. The resulting optimal paraffin impregnation gave blocks that were easy to cut, including the tissues that contained fat.

However, this study does not address all the possible issues concerning the effect of microwave-assisted tissue processing on histologic quality. For example, our study did not have the arm where microwave fixation was followed by conventional dehydration and clearing and again wax impregnation under microwave vacuum. In addition, we used vacuum in microwave histoprocessing, but not in conventional method since we do not have that facility in our setup. Therefore, the effects of vacuum and microwave heat in histoprocessing got clubbed together and cannot be distinguished from our results. Further studies are required to clarify these issues.

CONCLUSIONS

- Overall, the quality of microscopic tissues from the traditional processing and the microwave processing methods were identical. It was not possible to distinguish between the two techniques by studying the tissue sections. The overall morphology, overall staining, cellular outline, cytoplasmic and nuclear details, erythrocyte integrity and lymphocyte appearance of tissues processed by microwave method were comparable to or superior to that processed by the conventional method.
- The overall staining of the tissues where wax impregnation was done in microwave was better than conventional method.
- Thyroid and gastrointestinal tissues were better fixed in microwave method than the conventional method.
- Malignant lesions showed better histologic quality when dehydration, clearing and wax impregnation were done in microwave method.
- Microwave processing had several advantages over routine methods from the perspective of laboratory personnel. It eliminated the need for xylene in tissue processing. Use of formalin is optional as determined by the laboratory. There was a drastic reduction in the turnaround time without compromising the overall quality of the histologic sections. Microwave processing substantially shortened the time from specimen reception to diagnosis. It allowed 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 reagents used in the microwave method were much cheaper than those used in the conventional method and minimal amount of reagents were required. Microwave method can be used as a substitute for conventional method for routine work load of the lab. Since the advent of microwave histoprocessing it has been used routinely in the daily workload of the laboratory since the past 2 years.
- 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. Conventional processing produces peak laboratory activity in the mornings, whereas microwave histoprocessing has a continuous and even activity throughout the day, which is not easily accepted by laboratory staff.

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When the type of lesion was taken into account, malignant lesions showed significant difference in the processing in Protocol II ($p = 0.015$ sig) and Protocol III ($P = 0.026$ sig), where microwave processing was better [Figure 2]. The tissue sections had better cytoplasmic and nuclear details with good erythrocyte integrity and lymphocyte appearance than the conventional method. For other tissues of normal histology, non-neoplastic and benign lesions, there was no significant difference in the processing in

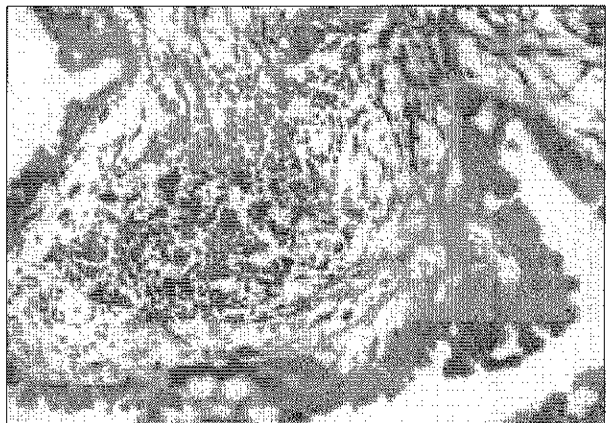

Figure 1: Excellent erythrocyte integrity - Protocol IV (H&E, ×400)

Table 2: The seven protocols used in the study

Steps	*P I*	*P II*	*P III*	*P IV*	*P V*	*P VI*	*P VII*
Fixation	MW	C	C	MW	C	MW	C
Dehydration	C	MW	C	MW	MW	MW	C
Clearing	C	MW	C	MW	MW	MW	C
Wax impregnation	C	C	MW	C	MW	MW	C
Duration	15 hrs 20 min	18 hrs 20 min	7 hrs 20 min	14 hrs 40 min	6 hrs 40 min	3 hrs	19 hrs

P = Protocol, MW = Microwave, C = Conventional

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Table 3: Comparison between microwave and conventional methods in all the protocols

Protocol	*Method*	*N*	*Mean*	*Std. Deviation*	*Z*
I	Microwave	31	20.7097	2.35504	1.272,
	Conventional	31	19.8387	2.84132	$p = 0.185$ ns
II	Microwave	31	21.3226	1.24866	1.876,
	Conventional	31	19.9677	4.26211	$p = 0.065$ ns
III	Microwave	31	21.4194	0.84751	2.482,
	Conventional	31	20.7742	1.14629	$p = 0.022$sig
IV	Microwave	31	21.4516	1.05952	1.769,
	Conventional	31	20.9677	1.27760	$p = 0.413$ ns
V	Microwave	31	21.2903	0.90161	0.394,
	Conventional	31	21.3548	0.98483	$p = 0.694$ ns
VI	Microwave	30	20.6333	2.28161	0.405,
	Conventional	30	20.4000	1.86806	$p = 0.672$ ns

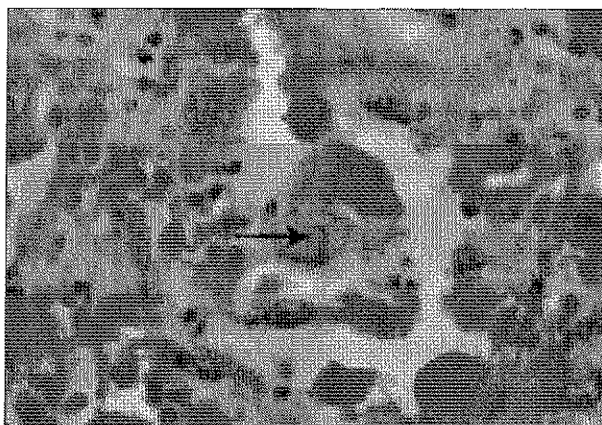

Figure 2: Prominent nucleolus (arrow) in melanoma cells- Protocol II (H&E, ×1000)

any of the protocols. The endoscopic biopsies processed along with the large tissues also produced excellent results with the same temperature control.

The microwave technique did not produce any deleterious effects on special stains. Periodic acid - Schiff, van Gieson, Congo red, Masson's trichrome, alcian blue, mucicarmine and reticulin staining pattern and intensities on tissue processed by microwave method could not be distinguished from those by conventional method.

Decalcification of bone with 5% formic acid using vacuum-microwave histoprocessor was studied. The final sections were excellent when compared with conventional processing with a drastic reduction in time from days to a maximum of 9 h.

DISCUSSION

A potential application of microwave energy in histotechnology was first recognized by Mayers in 1970.[5] This form of non-ionizing radiation produces alternating electromagnetic fields that result in the rotation of dipolar molecules such as water and the polar side chains of proteins through 180° at the rate of 2.45 billion cycles/second. The molecular kinetics so induced results in the generation of energy flux and continues until radiation ceases.

Boon *et al.* reported that it is possible to produce significant acceleration of tissue processing by introducing microwaves to the procedure.[2] Kok and Boon described a novel technique, where vacuum was combined with microwaving.[6] They have exploited extensively the fact that the boiling temperature of liquids featured in histoprocessing decreases when the liquid is placed in vacuum. Accordingly, vacuum was used to 'pull out' the molecules. Moreover, this can be done at relatively low temperatures, so that the tissue is protected against the negative effects of overheating. A similar vacuum-microwave histoprocessing technique was used in the present study.

A specially designed Milestone RHS-1 vacuum histoprocessor was used. The microwave unit had width of 20', depth of 27.3' and height of 22.8', with maximum power of 800 W, 230 V. The vacuum chamber had an infra-red sensor, a fully automated non-contact temperature control, an exhaust fan to extract vapors so that no toxic vapors are released to laboratory, a vacuum outlet to improve extraction of solvents from tissue and for wax impregnation and an auto start magnetic stirrer for homogenous temperature distribution within the solution eliminating 'hot spots'. For vacuum-microwave steps, an external vacuum module condenser was developed to collect the vapors from the RHS unit before they went into the vacuum module controller. The instrument was equipped with software regulating power output, temperature and time. The plastic cassettes, each one containing one tissue block, were put in one specially designed holder. The holder used could contain a maximum of 110 cassettes. The loaded holder was put in a glass container of 5 l capacity and kept in the vacuum chamber. The thickness of each of the tissue sections was of the order of 0.5 cm similar to that in previous studies,[7-9] so that the microwave excitation was virtually uniform throughout the tissue.

After each step, the volume of the tissue had decreased. The decrease in volume varies between different tissues and is similar to that of the conventional technique. However, the microscopical results of both the methods were excellent in concordance with that of Kok *et al.*[8] Microwave processing was less effective in processing large number of tissues. Therefore, the reagents had to be changed every third day when 110 tissue sections were processed at a time. Similar problem was encountered by Kok *et al.*[8] The microwave oven did not produce any 'hot spots' or the uneven heating encountered in smaller domestic microwave oven; mainly because of the magnetic stirrer kept beneath the rack, which provides an even field of irradiation. The magnetic stirrer is very important in the third step of wax impregnation. The third step should be carried out immediately, otherwise the molten paraffin gets solidified and the stirrer does not rotate, resulting in increase in pressure in the chamber, stopping the program. This was overcome by keeping the microwavable container with molten paraffin permanently in the hot air oven.

The present study compared the histologic quality of the slides processed by microwave method and the conventional method. The parameters and the ratings of the adequacy used were similar

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to those of Titford and Horenstein.[10] Fixation in microwave with 10% buffered formalin for 20 min at 50°C was compared with the conventional method. The findings were in agreement with those of Gordon.[11] There was no erythrocytolysis, indicating good performance in the fixation of cellular membrane as observed by Titford and Horenstein and Boon *et al.* There was no significant difference in the nuclear size and the chromatin pattern was reticular with good lymphocyte appearance scores, indicating good performance in the fixation of chromatin. These findings were similar to those of Titford and Horenstein. Excellent cytological preservation with good preservation of nuclear membranes, adequately clumped chromatin and well-defined cellular outlines noted in the present study were in agreement with those of Gordon.[11] Among the various organs processed, there was significant difference in the fixation of gastrointestinal and thyroid tissues, where the microwave method was superior ($P = 0.05$ sig and $P = 0.008$ hs, respectively). Other organs did not show any significant difference. In all the sections, the tissue architecture was well-maintained with no shrinking or spongy pattern. No crisp ethyl alcohol patterns of nuclear features were seen as observed by Boon *et al.* [8] No microwave-induced vulnerability such as condensation and shrinkage of surface tissue with nuclear pyknosis, loss of cytoplasmic details and lakes of red blood cells in the region of shrinkages was noted in contrast to those observed by Gordon who exposed tissues to microwaves in air. Therefore, there was uniform formaldehyde fixation. It was significant that microwave fixation followed by routine conventional method did not alter the tissue morphology and cellular details or the criteria for establishing malignancy. The major advantage of microwave fixation was the marked reduction in the time required for the fixation of tissue prior to dehydration and hardening, taking approximately 20 min when compared to hours in conventional method. Moreover, the microwave fixed tissues were more easily cut on the microtome as claimed by Bezahler.[12]

Dehydration and clearing were done in microwave at 70°C for 2 h. Macroscopically, the tissue blocks did not shrink during histoprocessing and paraffin was observed to have impregnated everywhere in the tissue. The paraffin blocks were easy to cut. Malignant lesions showed better histologic quality with microwave dehydration and clearing ($P = 0.015$ sig).

Wax impregnation alone was done in the microwave unit for 20 min for 31 tissues and compared with the conventional method. There was significant difference in the overall staining when compared with the controls ($P = 0.049$ sig), where 28 (90.3%) sections had uniform good staining. This may be attributable to the vacuum step introduced prior to wax impregnation, where the residual isopropyl alcohol in between the tissues is removed, permitting uniform wax impregnation. Irregular nuclear membrane, prominent nucleoli and mitotic figures in malignant lesions were also clearly evident in the tissue sections. Malignant lesions showed significantly better histologic features when wax impregnation was done in microwave method ($P = 0.026$ sig). Microscopically similar features were observed when dehydration, clearing and wax impregnation were combined in the microwave method. Overall, the quality of microscopic tissues from conventional processing and microwave processing methods were identical. It was not possible to distinguish between the two techniques by studying the tissue section.

In Protocol VI, all the steps were combined in microwave simulated method. Noteworthy was the complete absence of tissue shrinkage during histoprocessing. This was highly important for quantitative pathology, where objective measures were required, for example, for depth of invasion or volume of tumor. There was no significant difference between nuclear size and shapes, and staining characteristics were discernable. Similar findings were observed by Sivadas *et al.*[13] where alcohol, chloroform and wax were used for processing. However, cytoplasm stained moderately eosinophilic with no deeper eosinophilia as observed by them.

For routine purposes, it is often desirable to obtain the paraffin sections in a few hours, but this is not possible with the conventional method. In the present study, the microwave processing with Protocol VI met the above demand. Protocol VI, where all the steps of fixation, dehydration, clearing and wax impregnation were done in microwave, took a total processing time of 3 h. Thus, in this manner, it was possible to run several short cycles of about 3 h each during the working day so that stained sections were available on the same day as the specimens were received. Endoscopic biopsies were processed through a short cycle of 40 min using the same reagents. In our routine practice, we processed biopsies and resected specimens together, as such sampling error is not a problem with vacuum-microwave processing, which was noted by Suri *et al.*[14] But despite the advantages, microwave procedure is still not practiced widely.[15] This is mainly due to the established working schedule in the laboratories. With conventional processing, the workload is maximum in the morning, while microwave processing produces continuous even activity during the day, which is not easily accepted by laboratory staff. Therefore, in the routine setup of this laboratory, Protocol IV of microwave processing was found suitable, taking into consideration the working pattern of technical staff who worked daily from 9:00 am to 5:00 pm. All the specimens that reached the laboratory till 4:00 pm were grossed and fixation, dehydration and clearing were done in microwave on the same day. One of the laboratory officials had to stay after 5.00 pm to keep the tissues in molten paraffin wax where it remained overnight. The next day the sections were embedded and slides were made available to the pathologists for reporting by 2:00 pm. Even though the whole procedure takes 14:40 hours, the slides were ready for reporting next day afternoon. This was 1 day ahead when compared with conventional processing where the duration of processing was 19:00 hours and the slides for reporting were made available after 2 days.

Microwave processing had several advantages over routine method. It eliminates the use of xylene from the processing and also formalin as determined by the laboratory. Microwave processing substantially shortens the time from specimen reception to diagnosis without compromising the overall quality of the histologic section.

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Since isopropyl alcohol was also a dehydrating agent, processing with isopropyl alcohol and wax, a two-step procedure was done in microwave processor as suggested by Boon *et al.*[2] This method could save ethanol or is useful when ethanol is not available. However, the method described in the present study, a three-step procedure with methanol, isopropyl alcohol and paraffin combined with vacuum gave excellent results than the two-step procedure. Use of reagents was minimal in this method and the paraffin can be used several times. In the present study, the used isopropyl alcohol was discarded every third day. The resulting optimal paraffin impregnation gave blocks that were easy to cut, including the tissues that contained fat.

However, this study does not address all the possible issues concerning the effect of microwave-assisted tissue processing on histologic quality. For example, our study did not have the arm where microwave fixation was followed by conventional dehydration and clearing and again wax impregnation under microwave vacuum. In addition, we used vacuum in microwave histoprocessing, but not in conventional method since we do not have that facility in our setup. Therefore, the effects of vacuum and microwave heat in histoprocessing got clubbed together and cannot be distinguished from our results. Further studies are required to clarify these issues.

CONCLUSIONS

- Overall, the quality of microscopic tissues from the traditional processing and the microwave processing methods were identical. It was not possible to distinguish between the two techniques by studying the tissue sections. The overall morphology, overall staining, cellular outline, cytoplasmic and nuclear details, erythrocyte integrity and lymphocyte appearance of tissues processed by microwave method were comparable to or superior to that processed by the conventional method.
- The overall staining of the tissues where wax impregnation was done in microwave was better than conventional method.
- Thyroid and gastrointestinal tissues were better fixed in microwave method than the conventional method.
- Malignant lesions showed better histologic quality when dehydration, clearing and wax impregnation were done in microwave method.
- Microwave processing had several advantages over routine methods from the perspective of laboratory personnel. It eliminated the need for xylene in tissue processing. Use of formalin is optional as determined by the laboratory. There was a drastic reduction in the turnaround time without compromising the overall quality of the histologic sections. Microwave processing substantially shortened the time from specimen reception to diagnosis. It allowed 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 reagents used in the microwave method were much cheaper than those used in the conventional method and minimal amount of reagents were required. Microwave method can be used as a substitute for conventional method for routine work load of the lab. Since the advent of microwave histoprocessing it has been used routinely in the daily workload of the laboratory since the past 2 years.
- 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. Conventional processing produces peak laboratory activity in the mornings, whereas microwave histoprocessing has a continuous and even activity throughout the day, which is not easily accepted by laboratory staff.

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