Original Article

Comparison of Prostatic Tissue Processed by Microwave and Conventional Technique Using Morphometry

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ABSTRACT

Background and Objectives: **Rapid processing of histopathological specimens and decreased turnaround time is important to fulfill the needs of clinicians treating sick patients, so the present study was conducted to compare the time taken and quality of sections in processing of prostatic tissue by rapid microwave and conventional techniques using morphometry.**

Methods: **Four to five mm thick paired prostate tissue pieces of fifty cases of prostatectomy specimens were taken. One tissue piece of the pair was processed routinely overnight by conventional tissue processing and the other by microwave processing. Time taken for processing by both conventional technique and microwave technique was noted and compared. Then, both were stained with conventional method of hematoxylin and eosin staining and examined for histological typing and grading. Morphometric study was done on slides of prostatic tissue processed by both conventional and microwave technique.**

Result: **The prostatectomy specimens included both benign (86%) and malignant (14%) prostatic lesions in the age range of 46-85 years. The time taken for steps of dehydration, clearing and impregnation in microwave technique was significantly less as compared to histoprocessing done by conventional technique. Morphology, staining patterns of prostatic tissue processed within minutes by microwave technique, whether benign or malignant, were comparable to those sections which were processed in days using standard technique.**

Conclusion: **Domestic microwave oven can be used for histoprocessing to accelerate the processing with preservation of morphology and is cheaper than commercially available microwave ovens and processing time was considerably reduced from days to minutes.**

Keywords: **Prostate, Histological Technique, Microwave**

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Introduction

Specimens from operation theatre needs fixation and processing to preserve their structure, and eventually impregnated with an appropriate hardening substance to permit thin slices suitable for staining and microscopic evaluation. The basis for histoprocessing technique was laid more than a hundred years ago and has practically remained unchanged, until the invention of automated tissue histoprocessors. These have significantly shortened the processing time from several days to about one day (1). However this too means delay in report generation for one day or longer leading to delay in planning or institution of the treatment which is crucial in critically ill patients. Thus to reduce this turnaround time, microwave technology has been introduced in the field of histoprocessing and staining.

Microwaves are electromagnetic waves ranging in frequency from 300 MHz to 300 GHz. These waves can be used on their own or in combination with chemical fixation resulting in shorter processing time leading to increased workflow in laboratory as compared to conventional processing technique (2). It permits same day diagnosis for biopsy specimens (3, 4).

The present study was conducted to document the usefulness of microwave assisted tissue processing in prostatic tissue specimens over conventional histoprocessing comparing their histologic quality and the total turnaround time. Morphometry was done on both conventional and microwave processed histopathological sections to assess the cellular details in an attempt to improve the accuracy and reproducibility of histopathological diagnosis.

Materials and Methods

Fifty cases of prostatectomy specimens were included in the present study. Four to five mm thick paired formalin fixed prostate tissue pieces were taken. One tissue piece of the pair was routinely processed overnight by automated conventional tissue processor and the other by microwave processing using a domestic microwave oven.

In microwave histoprocessing technique (5), the tissue pieces were processed at 450 Watt, first in absolute alcohol for approximately 5 min or until the alcohol evaporated completely. Then the hardened tissue pieces were put in xylene (for clearing) for 1.5 min to reach a temperature of around 70 ^{0}C followed by keeping in liquid paraffin for 2 min. The jar with tissue in the liquid paraffin was removed from the microwave and subsequently embedded in paraffin wax as is done in conventional processing.

The other half of the material was processed with the routinely used conventional histoprocessing technique using automated tissue processor (5, 6). After embedding with paraffin wax, the tissue pieces processed by both microwave and conventional technique were subjected to routine sectioning (of approximately 5nμm thickness) and staining with Hematoxylin and Eosin stain (H & E) (6, 7).

The stained slides of prostatic tissue processed by both microwave and conventional technique were studied for routine histological examination using light microscope and diagnosis was made based on characteristic histopathological findings. Then morphometric study was done on the sections prepared from both the tissue processing techniques, using image analyzer. One hundred epithelial cells were randomly selected and measured in each case. The cells of interest were identified on the screen and the contours of their nuclear and cytoplasmic profiles traced manually. The parameters studied were mean nuclear area, mean cytoplasmic area, mean N/C ratio.

Results

The time required for histoprocessing, including dehydration, clearing and impregnation was 5, 1.5, 2 min by microwave technique and 480, 240

and 240 min by conventional tissue processing technique. Hence the processing time was reduced to 8.5 minutes by microwave technique as compared to 960 min by conventional technique (Table 1). On light microscopy, 43 out of 50 cases were diagnosed as benign prostatic hyperplasia (BPH), while 7 cases were of carcinoma (Ca) prostate (Fig. 1). Architectural grading using Gleason grading system was done in all cases of Ca prostate. We found that in sections processed by microwave technique, the overall tissue architecture, stroma, cell and nuclear morphology appeared to be similar to routinely processed sections. All the H & E stained sections of prostatic tissue processed by both histoprocessing techniques were subjected to morphometric study. Mean nuclear area, mean cytoplasmic area and mean N/C ratio was calculated and compared in all cases and found to be statistically insignificant.

Table 1- Comparison of time taken by various steps of histoprocessing by microwave and conventional processing techniques

Sample Number	Steps of histoprocessing	Average time taken by microwave technique	Average time taken by conventional technique	*P*- value
1	Dehydration	5.0 min	480 min	< 0.005
2	Clearing	1.5 min	240 min	< 0.005
3	Impregnation	2.0 min	240 min	< 0.005
	Total	8.5 min	960 min	< 0.005

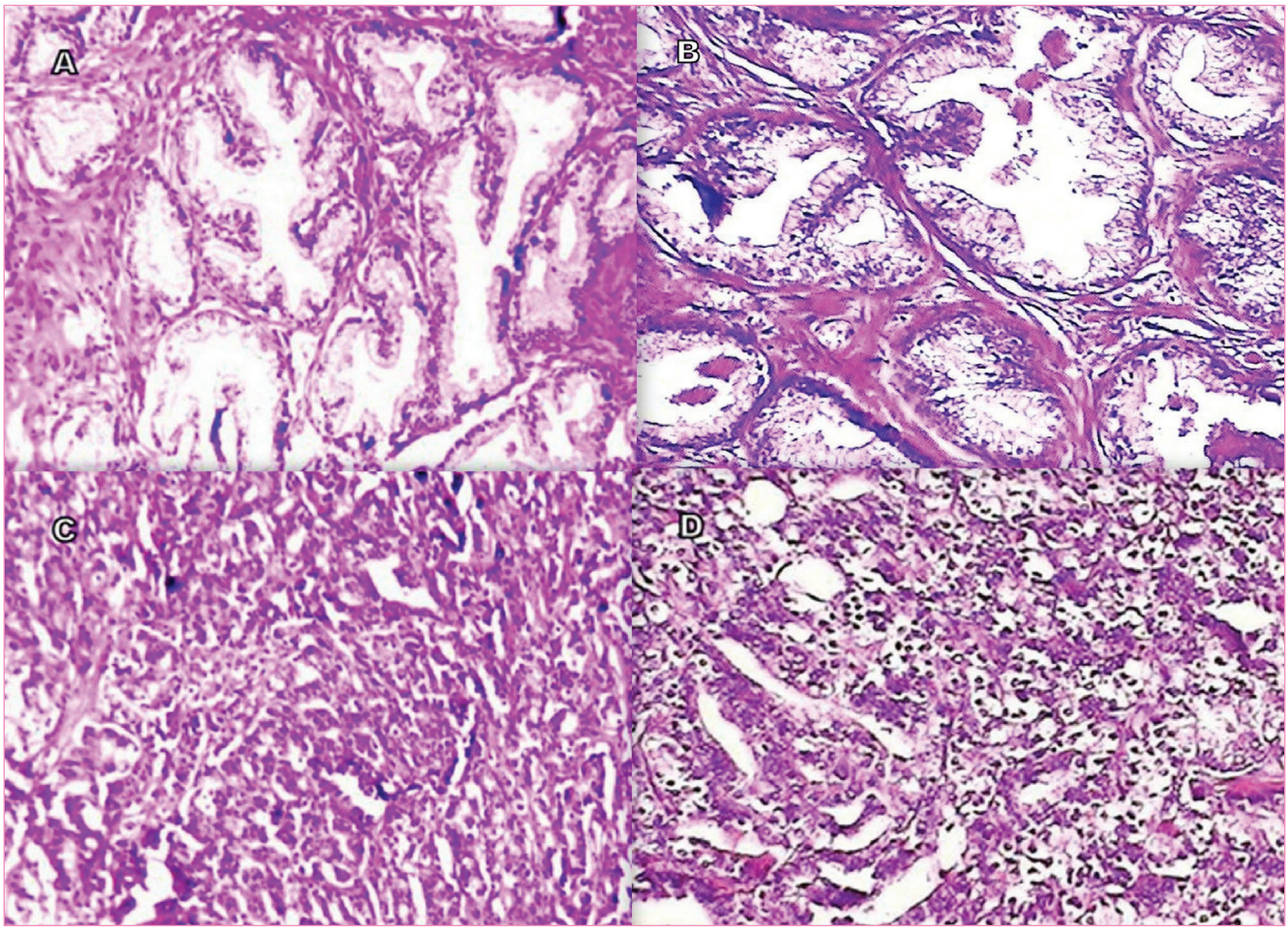


Fig. 1: Photomicrograph Showing **A)** Conventionally Processed Benign Prostatic Hyperplasia ×100 ; **B)** Microwave Processed Benign Prostatic Hyperplasia ×100; **C)** Coventionally Processed Carcinoma Prostate ×400 ; **D)** Microwave Processed Carcinoma Prostate ×400 (H & E)

In 43 cases of BPH processed by conventional method, nuclear areas were in the range of 21.02-61.36 μm^2 with a mean of 35.59±8.67 μm^2, whereas in 7 cases of Ca prostate nuclear areas were in the range of 62.42-95.23 μm^2 with a mean of 79.56±12.02 μm^2. In the tissues processed by microwave technique the nuclear areas in benign cases (N=43) were in the range of 20.83-62.85 μm^2 with mean of 34.80±8.21 μm^2 where as in 7 cases of Ca prostate nuclear areas were in the range of 63.52-81.06 μm^2 and mean was 72.85±7.14 μm^2.

Thus, mean nuclear area values in prostatectomy specimens were slightly higher by conventional method as compared to microwave processing method. However, this variation was statistically insignificant (*P*- value >0.05).

Out of a total of 50 cases processed by conventional method, in 43 cases of BPH the cytoplasmic areas were in the range of 66.41-194.46 μm^2 with a mean of 114.83± 26.08 μm^2, whereas in 7 cases of Ca prostate cytoplasmic areas were in the range of 84.47-141.50 μm^2 with a mean of 111.40±25.49 μm^2.

In the tissues processed by microwave technique, the cytoplasmic areas in benign cases (N=43) were in the range of 70.37-215.35 μm^2 with mean of 116.27±27.62 μm^2 where as in 7 cases of Ca prostate, cytoplasmic areas were in the range of 79.70-120.25 μm^2 and mean was 100.52±17.40 μm^2 (*P*- value >0.05).

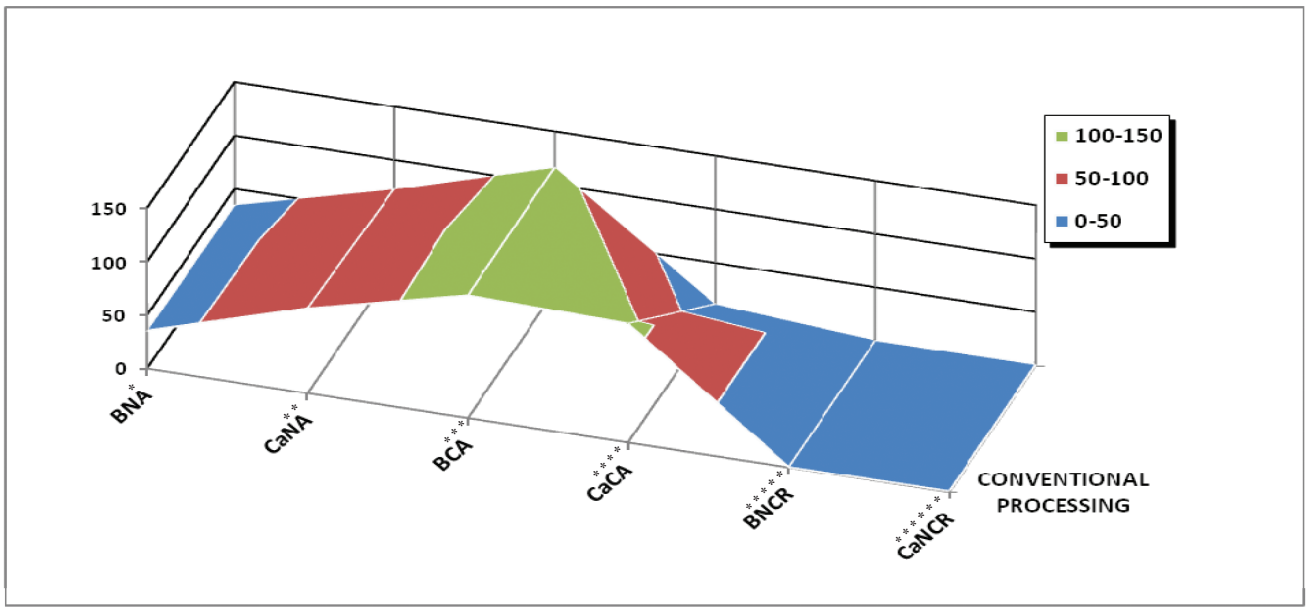
Similarly out of total 50 cases processed by conventional method, in 43 cases of BPH N/C ratio was in the range of 0.23-0.36 with a mean of 0.30±0.03, whereas in 7 cases of Ca prostate N/C ratio was in the range of 0.63-0.82 with a mean of 0.72±0.07.

In the tissues processed by microwave technique, the N/C ratio in benign cases was in the range of 0.24-0.38 with mean of 0.30±0.03 where as in 7 cases of Ca prostate N/C ratio was in the range of 0.64-0.80 and mean was 0.73±0.06. Thus, the variation in nuclear area, cytoplasmic area and N/C ratio processed by conventional and microwave techniques were statistically insignificant (Table 2) (Fig. 2).

Table 2- Comparison of nuclear area, cytoplasmic area, N/C ratio of cases of Prostatectomy specimens processed by conventional and microwave techniques

		Conventional Processing Method		**Microwave Processing Method**		
	Category	**Range**	**Mean ± SD (μm^2)**	**Range**	**Mean ± SD (μm^2)**	***P*- value**
Nuclear Area	Benign prostate	21.02-61.36	35.59±8.67	20.83-62.85	34.80±8.21	> 0.05
	Carcinoma	62.42-95.23	79.56±12.02	63.52-81.06	72.85±7.14	
Cytoplasmic Area	Benign prostate	66.41-194.46	114.83±26.08	70.37-215.35	116.27±27.62	> 0.05
	Carcinoma	84.47-141.50	111.40±25.49	79.70-120.25	100.52±17.40	
N:C Ratio	Benign prostate	0.23-0.36	0.30±0.03	0.24-0.38	0.30±0.03	> 0.05
	Carcinoma	0.63-0.82	0.72±0.07	0.64-0.80	0.73±0.06	

N/C ratio: Nuclear / Cytoplasmic ratio



*: BPH Nuclear Area, **: Carcinoma Prostate Nuclear Area,
: BPH Cytoplasmic Area, *: Carcinoma Prostate Cytoplasmic Area,
*****: BPH N/C Ratio ******: Carcinoma Prostate N:C Ratio

Fig. 2: Comparison of nuclear area, cytoplasmic area and N/C ratio in different categories

Discussion

Prostate is an important sexual organ in males. Development of hyperplasia and carcinoma in prostate, causing obstruction of urinary flow is a leading cause of morbidity and mortality in elderly males. The incidence increases with age, so early diagnosis is warranted in all such cases (7, 8).

In most pathological laboratories, turnaround time for tissue processing is approximately 24 hours to prepare slides of the surgical specimen for the pathologist to make a diagnosis. The surgical specimens received are processed overnight and evaluated by the pathologist the following afternoon. The goal of processing is to remove all water from the tissue through a series of graded alcohols and xylene, and then impregnate with paraffin wax. This allows the tissues to be embedded, sectioned and placed on a slide for staining and pathologists' review. This process has not changed for almost a century. In high-throughput laboratories, automation has become routine in the form of automatic processors, slide stainers and cover slippers. Several attempts have been made to further reduce the time for tissue processing for the last 2-3 decades using microwave technology.

Microwaves are non-ionizing radiation producing alternating electromagnetic fields that result in the rotation of dipolar molecules through 180° at the rate of 2.45 billion cycles per second (8, 9). A potential application of microwave energy in histotechnology was first recognized by Mayers in 1970 (9, 10). Since then, various studies have been conducted demonstrating the applications of microwaves across a wide spectrum of histologic procedures, including fixation and histochemical staining for light and electron microscopy, preservation of cryostat sections and in immunohistology (9, 11-14). Exposure of tissue to microwaves for shorter periods leads to marked acceleration of all these procedures, with greater consistency of results and enhanced histochemical and immunohistologic staining. In the present study, time required for histoprocessing of prostatic tissue by microwave technique (which included dehydration, clearing and impregnation) was reduced to 8.5 min, which was significantly less as compared to 960 min (16

h) by conventional histoprocessing technique. It was hereby observed that average time taken in microwave processing by most previous studies varied from 15 min to 67 min whereas the time taken in the present study was only 8.5 min (1, 3, 5, 9, 15-20).

Almost all the researchers have used automated temperature controlled microwave tissue processor except a few who used domestic microwave oven (5, 9, 17). Despite the variable equipments and reagents used, it was concluded that the time taken in histoprocessing by microwave technique was significantly less as compared to the time taken in conventional histoprocessing, without compromising the quality of sections.

In our study, prostatic tissue sections were subjected to identical conditions during staining with H & E. The stained slides were evaluated for staining characteristics, integrity of tissue architecture, as well as nuclear and cytoplasmic details. The microwave processed tissue sections were virtually indistinguishable from classically processed sections. Some of the microwave processed tissue sections were even better as regards to contrast, cell contours and staining characteristics. The improved turnaround time for prostate pathology reports enabled the reporting of almost all cases on the same day of receipt of the specimens.

Morphometric analysis was done using mean nuclear area, cytoplasmic area and N/C ratio. The results obtained in microwave processed sections were then comparable with prostatic tissues sections processed by conventional technique and found to be statistically insignificant in both BPH cases and Ca Prostate cases.

There is paucity of literature on morphometric analysis of prostatic tissue processed by microwave technique and its comparison with routinely processed prostatic tissue, therefore the present study was conducted.

On comparison of nuclear area in the present study with previous studies, our findings showed that mean nuclear area of prostatic tissue by microwave technique was comparable to that observed by conventional method. The findings of the present study were consistent with Boon *et al.* who measured the nuclei of various types of cells and found that the results of both microwave and conventional processing techniques were comparable (5). Kayser *et al.* did rapid microwave fixation, of healthy and deceased lung tissue, mediastinal and hilar lymph nodes, followed by conventional processing and compared it with conventionally fixed and processed tissue using morphometry. They observed that morphometric measurement did not reveal any difference in nuclear size of tissues which were microwave fixed as compared to tissue fixed and processed conventionally (21).

In our study, apart from nuclear area, two more parameters -cytoplasmic area and nuclear cytoplasmic (N/C) ratio were studied morphometrically. Mean cytoplasmic area by both the techniques was comparable. In addition variation in N/C ratio in prostatectomy specimens processed by microwave technique and conventional technique was not significant. We could not find any previous study, which had included cytoplasmic variables for differentiating benign cells from malignant cells.

Previous studies (22, 23) and also the present study on morphometrically aided diagnosis indicated that nuclear characteristics are most relevant indicators in distinguishing benign cases from malignant cases. However, the cytoplasmic parameters further help to refine the predictive power of discriminate function. The data on the relative contribution of cytoplasmic variables in characteristically neoplastic cells in previous studies is lacking. Therefore we could not compare these variables in the present study with other previous studies.

Cost of processing per specimen was also calculated to see cost efficiency; with regards to amount of time, chemicals, electricity, manpower used and was found to be much less on microwave

processing while using a domestic microwave.

Conclusion

A domestic microwave oven can be used for histoprocessing as a cost effective tool to reduce the processing time (which was reduced from days to minutes), with preservation of morphology without compromising the overall quality of histopathologic sections.

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