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Pathology

XYLENE FREE TISSUE PROCESSING WITH CONVENTIONAL MICROWAVE OVEN

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ABSTRACT

AIM: To analyze the effect of Xylene free tissue processing combined with microwave oven in microscopic examination.

MATERIAL AND METHODS: 25 Biopsy samples received in the Department of Pathology, Government Mohan kumaramnagalam Medical College , Salem was included in the study . Two bits were taken and one processed in conventional automated tissue processor schedule and other bit by xylene free method . The sections were stained by H & E and for immunohistochemistry. The effect of staining quality in both H & E and IHC was compared .

RESULTS : The slides were reviewed by two pathologist and scored . Significant results were obtained with same quality on both methods of processing.

CONCLUSION : Xylene free method when combined with conventional microwave oven can be used as an rapid and effective alternate to routine processing in particular with tumours for immunohistochemistry.

KEYWORDS : Microwave Processing. Conventional Processing, Xylene

INTRODUCTION

Routine tissue processing consists of dehydration, clearing and then embedding steps. Various chemical were used for the same. Alcohol are used as dehydrating agents in varied concentrations. Xylene was used as a substitute for aniline dyes and benzenes in 1950s. But this was found to have various health hazards in both acute(< 2 weeks) and chronic (> 1 year) . Exposure to xylene can occur via inhalation, ingestion, eye or skin contact.CNS depression is very common issue.Long term exposure can lead to memory loss known as "organic solvent syndrome (1) It has effects also in GIT, Skin and reproductive system

Processing with using oils such as groundnut oil,coconut oil and others using kerosene mixed with xylene to reduce the toxic effects of xylene had been tried but unsuccessful (2) Liquid dish washing detergent has also been used with some good results by Falkeholm *et al.(*3)

The time for routine processing was in various schedules varying from 8 hrs to overnight schedule . The fast processing techniques includes Frozen sections, Rapid processing schedule and the recent is usage of microwave oven. Microwaves are non-ionizing electromagnetic waves with frequencies ranging from 300 MHz to 300 GHz and all kitchen microwave oven operate at 2.45GHz . The use of microwaves also helps in tissue fixation . Cellular antigens are distinctly better preserved in tissues fixed by microwaves than by conventional cross-linking fixatives, and accelerate the polymerization of resins in electron microscopy.

Mayers suggested usage of this in tissue processing and and Login first reported its success. But it was Kok and boon from Netherlands and Anthony Leong from Australia to first apply it in the year 1985.(4,6)
Very few studies have studied the comparison of processing with routine protocol and hence present study was done to analyze the efficacy and results.

MATERIAL AND METHODS

Biopsy specimens both small and large biopsy samples received in the Department of Pathology, GMKMC Salem during December 2018 was included in our study and 25 cases were taken.

Bony hard tissue were excluded for this study .

The grossing was done after adequate fixation of 24 hours and 2 bits were taken from same representative areas as per grossing protocol. In small biopsy it was sepearated into two halves First bit was processed in Thermo Scientific STP 120-1 Spin Tissue Processor. Second bit was processed by microwave technique with reference to guidelines found in literature. After processing they were embedded in parafiin wax and sections were taken in Leica automated microtome.

Hematoxylin and Eosin stain was done in both set and numbered. They were analysed for various parameters including the effects of processing, staining qualities like clarity of nuclei and cytoplasmic staining.Two cases of carcinoma breast included was tested for ER and PR immunohistochemistry by path insitu reagents . Both were tested for quality of staining and allfred scoring was done

Microwave processing

IFB microwave oven SFC 25 L was used for the study. After manual processing in 50 % , 70% ,80% 90% isopropanol for 45 minutes each ,it was followed by 1 hours in absolute alcohol I and 1 hours in absolute alcohol II. The tissue placed in thick plastic cassettes were then taken in microwavable plastic wide opened containers containing molten wax . Paraffin must be added to the microwave in liquid form as microwave energy will not melt paraffin pellets. (5) As the alcohol gets vapourised by heat in microwave ovens clearing agents like xylene is not required .

It was processed at 70° power for 5 minutes . After allowing to cool for two minutes it was followed by 30° w power for five minutes for two times. The tissues was then embed in L –blocks in fresh molten paraffin wax.

Routine processing :A change of 50 % ,70% 80 % 90 % for 45minutes and 1 $^{1/2}$ hour of three changes in absolute alcohol was done. This was followed by Xylene for 30 minutes of two cycles. The tissue was then processed in three changes of paraffin wax for 1 hour each. The tissues were finally embedded in paraffin wax.

Section cutting and staining: Processed samples of both methods were cut by Leica automated microtome and they were dewaxed by placing the slides in Hot air oven at 60 degrees and then transferring slides to Xylene for 10 minutes followed by isopropyl alcohol for 10 minutes. Then after transferring in running tap water in a staining trough , the slides are stained in Meyer's Hematoxylin for 8-10 minutes. They were differentiated by 1 % acid alcohol and then blueing done in running tap water with lithium carbonate. They were stained in eosin for 10 seconds and slides taken to alcohol and xylene ,airdried and mounted with coverslips . The stained slides were compared for colour intensity and uniformity. Nuclear details clarity and cytoplasmic components were too compared .

Immunohistochemistry

Two cases were taken for ER and PR receptor study.Deparafinised sections taken and antigen retrieval done in microwave for 2 changes of 5 minutes in Citrate buffer. Then quenching done by hydrogen peroxide in alcohol. Primary antibodies added and kept in moist environment. Secondaries and then chromogen were added. Mounted slides were studied for comparision

Stained sections was graded by two pathologist based on the parameters noted in Table (1)

Table -1 Scoring system

Sl No	Quality	Score I	Score 0
1	Tissue Preservance	Good	Poor
2	Cytoplasmic staining	Adequate	Inadequate
3	Nuclear Staining	Adequate	Inadequate
4	Clarity of staining	Crisp	Dull
5	Uniformity of staining	Uniform	Irregular

Observation and Results : After completion of processing and H& E staining the slides were reviewed by two pathologist and scored as in Table (1) . Individual scores given to integrity of tissue ,distinctness of Nucleus, cytoplasm and clarity of stain. Table 2

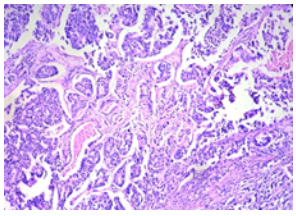

Fig 1. CA breast- routine processing

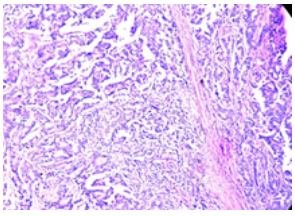

Fig 2.CA breast by Microwave processing

Table:1.Scoring of results (n = 25)

Score	Observer I		Observer II	
	Conventional	Microwave	Conventional	Microwave
5	17	16	19	19
4	5	6	3	4
3	3	3	3	2
2	0	0	0	0
1	0	0	0	0
0	0	0	0	0

The results were comparable in both methods as in study done by Mahesbabu et al (8) (Fig 1 and 2)In IHC , Allred scoring was done . It was found intensity scoring was more for both ER and PR by microwave processed slides.(Fig 3 and 4)

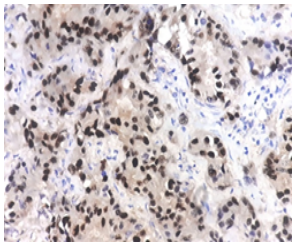

Fig 3. ER IHC by conventional

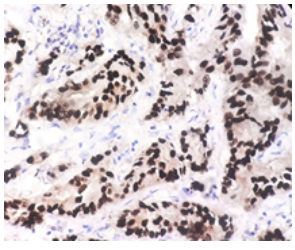

Fig 4 ER by Microwave

DISCUSSION

Histopathological tissue processing is a time consuming procedure with no marked changes for long years unlike in the field of fixation and microscopy. Tissues by fixation formalin type fixatives are by property of diffusion and are hardened by cross linking and denaturation of proteins. On subsequent heating this results in lesser tissue damage (6)

Morales *et al.*(7) found that in microwave processed tissues the epithelium was of better quality, while the stroma had a slightly different appearance, in that it appeared to be slightly more condensed focally . But in our study no marked difference in stroma and epithelium made.

Microwaves causes uniform heating of the tissue matter against irregular heating from outside in in conventional methods and helps better impreganation . This is because of the oscillation of the molecules and hence penetration of the solvent. By oscillation the collision may cause heat and causing kinetic motion with increased penetration.

Few studies have been conducted with xylene free processing and others on microwave processing. Present study combines the routine processing free of xylene and combined with microwave which has not been done. This helps in reducing the processing time markedly of clearing and impregnantion. Also the technical skill required in complete microwave dehydration is not here. The results are comparable with other methods followed and with increased intensity scoring with immunohistochemistry compared to traditional methods. Thus this method is an Non hazardous and Time effective in tissue processing with same to better results .

CONCLUSION

With recent updates in technology of tissue processing with microwave oven which has found way in many other industries years back can be utilized with comparable results in Quality but less hazardous and time effective .

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