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Original Research Article

Technical Modification and Enhancement of Staining Using Kitchen Microwave Oven

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ABSTRACT

Introduction: Good sample preparation and staining is the basis for successful interpretation of histopathological specimen. Staining of tissue specimens is based on two main factors namely diffusion of dyes into the cells and binding of the dyes to the substrate. Application of heat while staining hastens the staining procedure as it reduces the viscosity of the dye thereby increasing the diffusion of dye into the tissue sections. Hence, in this study we used inexpensive kitchen microwave oven with temperature control for microwave staining to compare with routine staining.
Aim: To assess the technical modification and enhancement of tissue staining by using kitchen microwave oven and to determine whether it can replace routine staining.
Materials and Methods: 100 surgically resected breast specimen blocks were taken. Two sections of each tissue blocks were mounted on two differently coded slides and stained with routine H&E (coded green) and microwave H&E (coded red) staining. The slides were evaluated by a pathologist totally blinded to the color coding.
Result: Microwave staining reduces the staining time by one-third. Cellular details, nuclear details and staining characteristics were not compromised in microwave staining rather were of good grades.
Conclusion: Microwave irradiation accelerates as well as enhances the staining of histopathological sections as compared to routine staining thereby, decreasing staining time for the reporting of histopathology. Thus, microwave can be used as a valuable adjunctive tool for staining and as an alternative to routine staining without compromising on the overall quality of the staining.

Keywords: Kitchen microwave oven, Microwave H&E staining, Routine H&E staining.

INTRODUCTION

Good sample preparation and staining is the basis for successful interpretation of histopathological specimen. [(1,2)] Staining of tissue specimens is based on two main factors namely: 1. Diffusion of dyes into the cells, which is a physical process.2. Binding of the dyes to the substrate, which is both physical and chemical.

Generally, the procedure of staining is tedious and requires hours for its completion. It is a known fact that the application of heat while staining hastens the staining procedure as it reduces the viscosity of the dye thereby increasing the diffusion of dye into the tissue sections. [(3,4)] Hence, microwave when used in staining helps in hastening staining along with enhancing the quality of staining as it provides uniform heating.

The microwave oven was invented only in 1945. It was first applied in histopathology for fixation in 1970 and in 1985 for tissue processing but today they are commonly used for performing simple

procedures such as specimen stabilization, staining, epitope retrieval and some decalcification procedures. Microwave is non-ionizing electromagnetic waves with a frequency ranging from 300 MHz to 300 GHz corresponding to the wavelength of 1 m to 1 mm, respectively. The kitchen microwave oven operates at 2.45 GHz with a corresponding wavelength of 12.2 cm. [(3-5)]

The basic functioning of microwave is based on the fact that each molecule of water has one big atom of oxygen attached to two little atoms of hydrogen and has a positive charge and a negative charge. Their penetration depth is dependent on the electric conductivity of the medium. Upon penetration into tissues, the energy is absorbed by the molecules. In the oscillating electric fields produced by microwaves irradiation, the dipolar molecules like water are forced to vibrate. Some of the acquired rotational energy is transferred to the random motion upon collision with other molecules. This induced kinetic movement produces instantaneous heat. This heat production increases the diffusion of the reagents and thereby enhances the staining as well as decreases the staining time. Unlike conventional heating, microwave heating is from within (internal heating) and its effect occurs throughout the material being irradiated. Microwave not only enhances the diffusion of the dyes but also enhances the binding of the dyes to the substrate. [(1-5)] This results in substantial reduction in tissue staining time and permitting faster diagnosis for a variety of types of tissue biopsy specimens.

Microwave irradiation is applied not only for accelerating routine stains but also for special, metallic, as well as immunofluorescent, immunohistochemical stains for both light and electron microscopy. [(4-11)] Laboratory microwave is highly preferred for histopathology as it offers many advantages, but is expensive. Hence, in this study we used inexpensive kitchen microwave oven with temperature control for microwave staining to compare with routine staining.

Hypothesis-Application of heat while staining of histopathological sections accelerates the staining procedure.

Alternate Hypothesis- Microwave irradiation accelerates as well as enhances the staining of histopathological sections as compared to routine staining.

Research Question- Do the technique modification of routine H&E staining by using microwave enhances the H&E staining of histopathological tissue and decreases the laboratory staining time for the reporting of histopathology.

Aims: To assess the technical modification and enhancement of tissue staining by using kitchen microwave oven and to determine whether it can replace routine staining.

Objectives: 1. Assess the time required for microwave staining as against the routinely stained specimen. 2. Assess the quality of the microwave stained tissue specimens as against the routinely stained specimen. 3. Evaluate the reliability of microwave staining using a kitchen microwave oven as against the routine staining.

MATERIALS AND METHODS

The present study titled, "Technical modification and enhancement of staining using kitchen microwave oven" was carried out in the Department of Pathology, Jawaharlal Nehru Medical College, Sawangi (M), Wardha over a period of 2 years from 2017 to2019. The study design was observational and cross-sectional. The study included 100 surgically resected breast tissue specimen received in the histopathology section of the Department of Pathology. The study was approved by institutional ethics committee.

Inclusion criteria: All surgically resected breast tissue specimens (benign and malignant).

Tissue blocks of 100 breast tissue specimen were selected. Two sections of each tissue block were cut and mounted on two differently coded slides. The slides were kept on hot plate to melt the wax and transferred in jar containing xylene which was subsequently stained with H&E. The

stained slides in each group processed by both microwave (coded red) and routine (coded green) methods were evaluated by a pathologist totally blinded to the color coding. The following parameters were assessed: 1)Time factor, 2)Cellular details, 3)Nuclear details and 4)Staining characteristics

Table 1: List of criteria evaluated by the pathologist

Criteria
Cellular details
Size of the cell
Cellular outline clarity
Cytoplasmic details
Nuclear details
Size of the nucleus
Clarity of the nucleus
Clarity of the nucleoli
Clarity of nuclear membrane
Clarity of the chromatin
Staining characteristic

Grading: good (+++), average (++) and poor (+)

Materials: 1) Biopsy specimens and tissues of breast received in Pathology department of JNMC. 2) H & E stain. 3) Kitchen microwave oven (LG- 700 W).

Methods: Histopathological section of breast tissue stained by routine H & E stain and microwave H & E stain were studied and compared.

Materials required for routine H&E Staining (Haematoxylin and Eosin):

- Harris Haematoxylin stain:
- Eosin stain: Eosin Y 1.0% solution in distilled water
- Xylene

Staining protocol for routine H&E Stain (Haematoxylin and Eosin stain): [(12)]

- Deparaffinize/ Dewax sections in xylene, 3 changes for 10 minutes each.
- Hydrate dewaxed sections through descending grades of alcohols to water.
- Running tap water
- Remove fixation stains if necessary.
- Stain with Harris's haematoxylin for 5 minutes.
- Wash well in running tap water until sections become blue for 5minutes.
- Differentiation is done in 1 % acid alcohol for 5-10 seconds (1 % HCL in 70 % alcohol).
- Sections are washed well in tap water until it is blue again (10-15 minutes).
- For 1 minutes, stain in 1% Eosin Y.
- For 1-5 minutes, wash in running tap water.
- Dehydration is done through 90 % alcohols and two changes of absolute alcohol.
- Clearing is done in xylene for 5 minutes.
- Apply cover slip with mounting medium DPX.

Interpretation- Nucleus was stained blue (basophilic) whereas cytoplasm was stained with varying shades of pink (eosinophilic).

Microwave H&E Stain (Haematoxylin and Eosin stain):

The kitchen microwave oven used in this study was operated at the output of 700 W throughout the study. The pretreated slide for microwave staining was placed on a microwave rectangular glass bowl containing tap water in the outermost rotating table of the microwave. For dehydration, the slides were first treated with 100% isopropyl alcohol. The tissue sections on slides were flooded with reagent and dyes. Dipping in 1% acid alcohol and eosin was done as per routine staining without microwave irradiation. To avoid slides from becoming warm, tap water in the rectangular glass bowl was periodically changed. Use of xylene within the microwave was completely avoided within the microwave, as they are highly combustible.

The optimum temperature for most metallic stain is 55-60 degree Celsius and for metallic stain between 75-95 degree Celsius. [(1)] However, the exact temperature at which the kitchen microwave oven was operated was not assessed as the kitchen microwave oven we used in the study does not have temperature control option. Hence, we took temperature control methods to counteract the excess temperature with the

help of tap water in the glass bowl over which the slides to be stained were placed.

Statistical Analysis

The result obtained were entered in Microsoft excel work sheets after which it was statistically analyzed using Student's unpaired t-test and Chi-square test. P value <0.05 was considered statistically significant.

Image 1: Kitchen microwave oven

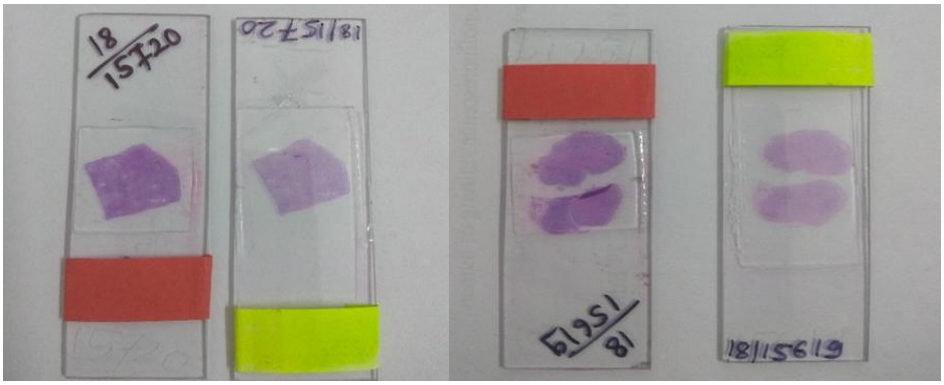

Image 2: Microwave H&E stained slides (red color)
Routine H&E stained slides (green color)

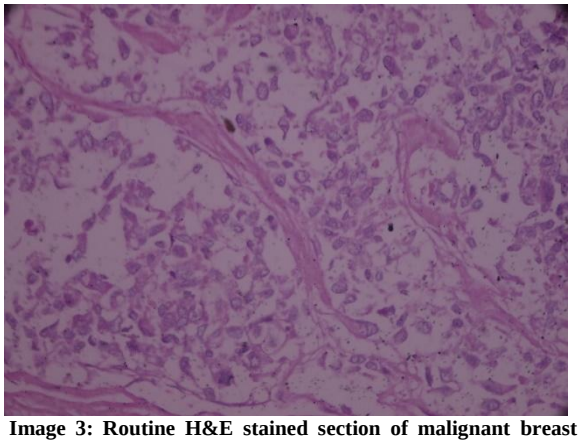

Image 3: Routine H&E stained section of malignant breast specimen(40X)

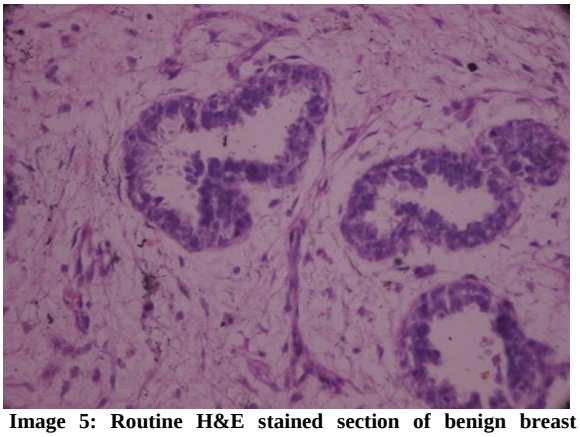

Image 5: Routine H&E stained section of benign breast specimen (40X)

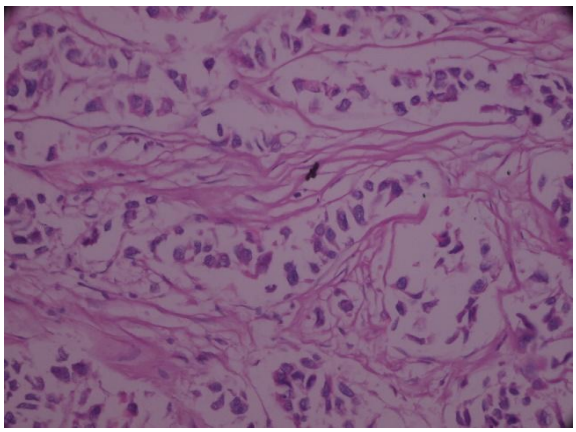

Image 4: Microwave H&E stained section of malignant breast specimen(40X)

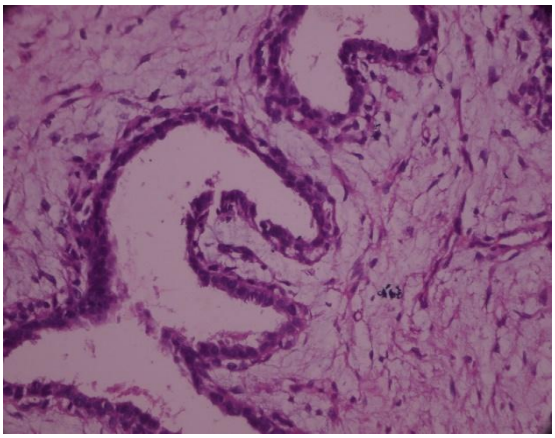

Image 6: Microwave H&E stained section of benign breast specimen(40X)

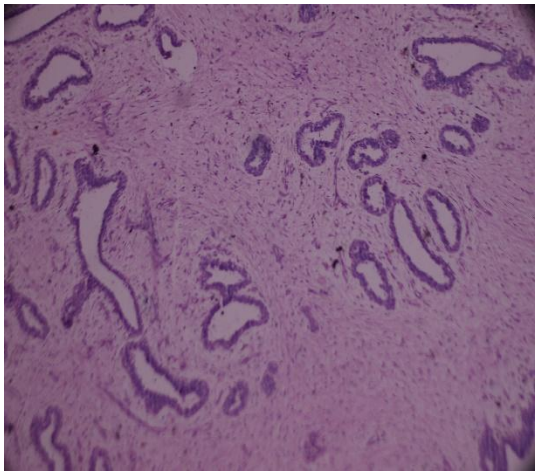

Image 7: Routine H&E stained section of benign breast specimen (10X)

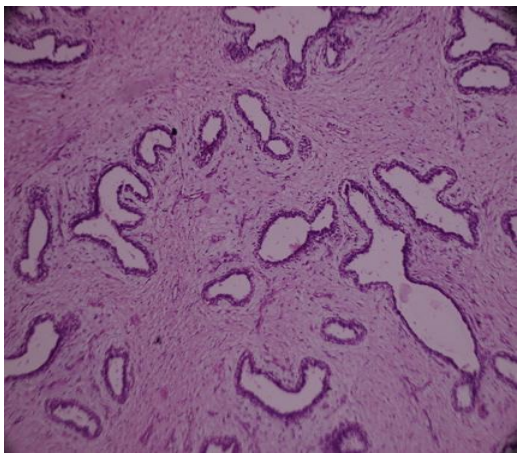

Image 8: Microwave H&E stained section of benign breast specimen (10X)

OBSERVATION AND RESULT

The present study titled, "Technical modification and enhancement of staining using kitchen microwave oven" was carried out in the Department of Pathology, Jawaharlal Nehru Medical College, Sawangi (M), Wardha over a period of 2 years from 2017 to2019. A total of 100 pair of slides of breast tissue specimen were stained for H & E. The stained slides in each group processed by both routine (coded green) and microwave (coded red) methods were evaluated. The time taken to stain the slides by both routine and microwave methods was calculated. The stained slides were graded for cellular details, nuclear details and staining characteristics, the results of which are as follows.

Time Factor

Table 2: Time factor forroutine and microwave H&E staining methods.

Reagent	Routine	Microwave
100% IPA	5min	3 min
Water	5min	3 min
Haematoxylin	5min	1min
Water bath	5min	1min
Acid Alcohol	10 sec	1dip
Water bath	15 min	1min
Eosin	1 min	1min
Alcohol	1 dip	1dip
Xylene	5 min	5 min
Total time	Approximately 41 min	Approximately 15 min

t= 4.36, p-value=0.031, Significant

In the present study, it was observed that the time required for microwave H&E staining was much shorter which was approximately 15 minutes than the routine H&E staining which took approximately 41 minutes. The time taken for microwave H&E staining is about one-third of the time taken for routine H&E staining. The time factor for microwave H&E staining was found to be statistically better than routine H&E staining (p-value <0.05).

Cellular Details

Table3: Grading of routine and microwave H&E stained sections based on cellular details

Cellular details	Routine H&E	Microwave H&E
Good	35%	65%
Average	58%	34%
Poor	07%	01%

χ^2= 19.76, p-value= 0.0001, significant

Out of total 100 pair of slides of breast tissue stained with routine and microwave H&E, cellular details of majority (58%) of routinely stained sections were graded as average, followed by good (35%) and poor (07%) grade as compared to the microwave stained sections, which were graded as good in majority (65%) of the cases, followed by average (34%) and poor (01%) grade.

The cellular details for microwave H&E staining was found to be statistically better than routine H&E staining (p-value <0.05).

Nuclear Details

Table4: Distribution of cases of routine and microwave H&E stained sections based on nuclear details.

Nuclear details (Size of the nucleus, clarity of nucleus and nuclear membrane)	Routine H&E	Microwave H&E
Good	36%	62%
Average	60%	35%
Poor	04%	03%

χ^2= 13.62, p-value= 0.0011, significant

The nuclear details (the size of the nucleus, the clarity of the nucleus and the clarity of the nuclear membrane) of the routine stained sections were graded as average in majority of the cases whereas the nuclear details of microwave stained sections were graded as good in majority of the cases.

The clarity of the nucleoli and clarity of the nuclear chromatin of both routine stained sections and microwave stained sections were graded as average in majority of the cases.

The nuclear details for microwave H&E staining was found to be statistically better than routine H&E staining (p-value <0.05).

Staining Characteristics

Table5: Staining characteristics of routine and microwave H&E stained sections.

Staining Characteristics	Routine H&E	Microwave H&E
Good	78%	89%
Average	22%	11%
Poor	0 %	0 %

χ^2= 19.76, p-value= 0.0001, significant

The staining characteristics of both routine and microwave stained sections were predominantly graded as good but the percentage of microwave stained sections graded as good was higher than routine stained sections by 11 %. The staining characteristics for microwave H&E staining was found to be statistically better than routine H&E staining (p-value <0.05).

Table 6. Statistical values of H&E staining

Criteria	p- value
Time factor	0.031
Cellular details	0.0001
Nuclear details	0.001
Staining characteristics	0.036

DISCUSSION

Obtaining good histological images for successful interpretation of tissue specimen of histopathological specimen is largely governed by good sample preparation and good staining. Microwave irradiation has been useful for both sample preparation and staining. The application of kitchen microwave oven used in this study for staining greatly accelerated the staining procedure.

In the present study, it was observed that the time required for microwave H&E staining was shorter than the routine H&E staining. Similar finding was also observed in the studies conducted by Mukunda et. al, Leong et. al and Cluggage at.al. [13-15] This is because microwave irradiation accelerates the staining by enhancing the diffusion of dyes into the tissue sections. Staining methods that normally take minutes can be done in a microwave oven in seconds, those that take hours can be done in minutes.

The cellular details of majority of the slides stained with microwave H&E in the present study were of good grade (65%) as compared to routine H&E (58%) which were of average grade. Similar finding was also observed in the study conducted by Mukunda et.al. [13]

The present study showed that the nuclear details (the size of the nucleus, the clarity of the nucleus and the clarity of the nuclear membrane) of the microwave H&E stained sections were graded as good in majority of the cases whereas the nuclear details of routine H&E stained sections were graded as average in majority of the cases.

The staining characteristics of both routine and microwave stained sections were graded predominantly as good but the percentage of microwave stained sections graded as good was higher than routine stained sections by 11 %. The staining characteristics for microwave H&E staining was found to be statistically better than routine H&E staining (p-value <0.05).

The above findings were similar with the findings of study conducted by Mukunda et.al. [13] The enhancement of staining in microwave H&E staining can be explained by the fact that microwave irradiation not only accelerates the diffusion of dye into the cells but also it enhances the binding of dye to the substrate.

The clarity of the nucleoli and clarity of the nuclear chromatin of both routine stained sections and microwave stained sections were graded as average in majority of the cases. This may be because for clear visualization of nucleoli and nuclear chromatin light microscopy with H&E stain is not the method of choice and requires specialized method such as electron microscopy.

Several studies have shown that microwave could be operated at and above 350 W wherein the time is in turn reduced to seconds. [15,16] The study by Mukunda et. al operated the kitchen microwave at the lowest output of 100 W. In the present study, we used the 700 W kitchen microwave oven. [13]

The time factor, cellular details, nuclear details and staining characteristics of the microwave H&E stained sections were found to be statistically better in comparison to the routine H&E stained sections with the p-value of 0.031, 0.0001, 0.001 and 0.036 respectively. These findings are similar to the findings of the study conducted by Mukunda et.al. [13]

In the present study, it was observed that kitchen microwave oven when used along with temperature control measures not only hastens the staining procedure thereby reducing the time, but also enhances the staining giving a better histological image. This can be particularly useful when there is a need of urgent histopathological reporting.

CONCLUSION

Microwave irradiation accelerates as well as enhances the staining of histopathological sections as compared to routine staining thereby, decreasing staining time for the reporting of histopathology. Thus, microwave can be used as a valuable adjunctive tool for staining and as an alternative to routine staining without compromising on the overall quality of the staining.

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