Original Article

Evaluation of Efficacy of Microwave Staining over Conventional Staining in Replicating Tissue Architecture: A Prospective Study

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INTRODUCTION

Incredible development of diagnostic methodologies has emanated over the period of years, of all nevertheless histopathological diagnosis endures an unassailable status in diagnosis of various lesions either in early or late stage. Despite all technical upgradation in the field of diagnostic pathology, the age-old process of staining tissue samples cannot be replaced. Histopathological processing and staining is an inordinately sensitive technique and tedious procedure. Staining of a tissue section is consecutively pretentious by various factors, all-encompassing of pressure, temperature, and pH. Various revisions have been made in recent years to streamline histopathological techniques. Use of microwave is one such annexation, which is likely to downturn the staining time compared to that of conventional technique.

ABSTRACT

Introduction: The use of microwave in the field of diagnostic pathology has gained a huge response in recent times. Use of domestic microwave ovens in the same is being widely studied. Unveiling the use of the microwave in improving the staining quality of tissue sections in the field of pathology can aid in precise diagnosis of complex conditions. Aim: The main aim of this study was to study the efficiency of microwave staining to reproduce the tissue architecture compared to that of conventional staining techniques. Materials and Methods: Thirty different tissue blocks (including 10 mucocele tissue blocks) were used to prepare 30 pairs of slides for three different stains, namely hematoxylin and eosin, Van Gieson’s, 0.1% toluidine blue and periodic acid–Schiff and 10 pairs of slides for mucicarmine stain. From each pair of slides, one slide was stained routinely, and the other was stained inside a microwave. Two pathologists evaluated the stained slides, and the results so obtained were analyzed statistically. Results: Microwave staining considerably cut down the staining time from hours to seconds. Microwave staining showed no loss of cellular and nuclear details, uniform staining characteristics, and was of excellent quality. Conclusion: The microwave stained slide showed no significant difference in terms of cell outline clarity, cytoplasmic staining, nuclear outline, nuclear staining, nuclear chromatin, and staining intensity compared to that of routine staining method, and a significant difference was observed in the total staining time consumed by all the stains that were used.

KEYWORDS: Conventional microwave, microwave staining, mucicarmine, routine staining

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Microwave was discovered by Percy Spencer in 1945.[1] Microwaves have ratified their application in a number of fields, of which three variants of microwave devices have been used in histopathology: (1) microwave devices explicitly contrived and certified for medical use, (2) commercial-grade microwave, which is rehabilitated for laboratory/clinical use, and (3) consumer household microwave units reformed for laboratory use.[1] Microwave devices adopted in histopathological laboratory work are based on the principle that microwaves thus generated impel to warm the tissue section from within, thereby facilitating faster penetration of dye molecules entailing to the reduction in staining time and improved staining quality.[2] This study aimed to compare the efficacy of domestic microwave oven in staining oral tissue samples compared to that of routine staining methods used, wherein we have used 30 normal mucosal samples and 10 mucocele samples. Routine processing methods were followed, and two sections of each block was made. Ten pairs of sections were stained using four stains, namely hematoxylin and eosin (HE), toluidine blue (TB), periodic acid–Schiff (PAS), and mucicarmine (MUC). One slide of each pair of section was stained by routine method, and the other was stained in microwave by following the protocol given by Mukunda et al.[3] The stained sections were then double-blinded and grading was done for six parameters by two observers.

**Aim**

The main aim of this study was to check the following:

1. Ability to use commercial microwave device in histopathological procedures
2. Efficiency of microwave staining over conventional staining in reproducing the tissue architecture
3. Comparison of the time consumed in case of microwave staining and conventional staining technique

**Materials and Methods**

Forty formalin-fixed routinely processed tissue blocks were used from the archival for this study. Normal and mucocele blocks from the archival material were selected based on the stains used in the study. The sole purpose of including mucocele sections was to show mucin using MUC stain. We used routinely used equipment and reagents for staining along with a basic model of conventional microwave oven.

Selected tissue blocks were sectioned so as to provide two bits of tissue sections from each block. Each section was mounted on two differently labeled slides. The slides were then placed on a hot plate until all the wax melted and then transferred to Coplin jars containing xylene. After 10 min, one slide from each pair was sent for routine staining, whereas the other slide was stained in a kitchen microwave oven. Four different stains, namely HE [Table 1], TB [Table 2], PAS [Table 3], and MUC [Table 4], were used in this study, all of which followed the same aforementioned preliminary procedure. Routinely stained slides were coded in red color, and microwave-stained slides were coded in blue color throughout the study. Two pathologists who were unaware of the color coding graded the slide based on the parameters given in Table 5. Routine staining was carried out in regular method, and for microwave staining, protocol given by Mukunda et al.[3] was followed.

The microwave was operated at 100 W during the entire study. The slides coded with blue color were placed in a petri dish containing water in the rotating table of the microwave. The tissue sections on the slide were flooded and replenished with appropriate reagents and dyes using a pipette. All steps except for dipping in 1% acid alcohol and eosin (only in few) were carried out without microwave irradiation. The water so present in the petri dish was frequently changed to avoid it becoming warm.

**Results**

A total of 20 pairs of slide were stained each for HE [Figure 1] (HE staining carried out by microwave method and routine method [×40]), TB [Figure 2] (0.1% TB staining performed by microwave method [×40]) and routine method [×40]), PAS stain [Figure 3] (0.1% PAS staining done by microwave method and routine method [×40]), and MUC [Figure 4] (MUC staining carried out by microwave method and routine method [×40]) were analyzed, and grading of the same was done based on the parameters mentioned in Table 5.

| Table 1: Microwave and routine staining protocol for hematoxylin and eosin |
|-------------------------------|---|---|
| Reagent                      | Routine | Microwave |
| 100% Isopropyl alcohol       | 15 min | 3 min    |
| Water                        | 10 min | 3 min    |
| Hematoxylin                  | 10 min | 1 min    |
| Blueing                      | 10 min | 1 min    |
| Acid alcohol                 | 1 dip  | 1 dip    |
| Water bath                   | 10 min | 1 min    |
| Eosin                        | 1 dip  | 1 dip    |
| Alcohol                      | 1 dip  | 1 dip    |
| Xylene                       | 10 min | 10 min   |
| Total time                   | Approximately 1 h | Approximately 20 min |

**Table 1: Microwave and routine staining protocol for hematoxylin and eosin**
Following which, \( \kappa \) statistics was performed to check for interobserver variability and \( \chi^2 \) test was performed to check if any significant difference was evident between routine staining and microwave staining with \( P \) value set at <0.05. The values thus obtained are present in Table 6.

The results in our study showed that no statistically significant difference was observed in staining characteristics, thus, produced in both microwave and routine staining but the fact that the time consumed has reduced drastically portrays the importance of microwave staining in routine histopathological protocol followed.

**DISCUSSION**

Staining of a tissue section is not a tranquil job. The eminence of staining is consecutively affected by various factors. Factors affecting the staining ability of a reagent, either directly or indirectly, include nature and pH of dye, affinity of dyes, concentration of dye, influence of fixative agent used, and nature of bond between dye and protein. Staining of a tissue section essentially reckons on dye–tissue or reagent–tissue interactions.[4] In general, if a tissue has immense affinity toward a tissue, it gets stained vehemently. This affinity sequentially depends on factors, which can either expedite or impede the movement of the reagent.[5] Another major parameter is the competence of the dye to enter into the tissue section and conceal itself in it, which is a physicochemical process.[6] Microwaves can act in both these states, facilitating rapid staining of tissue specimens.

Microwave-based tissue processing has been studied in a variety of fields since 1970. It has accomplished a colossal retort as processing technique for paraffin tissue sections, mainly wielded for immunohistochemical techniques.[7] Mayers was the first to develop microwave-assisted fixation of tissue samples in the year 1970, whereas Rogin confirmed the application of microwave
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Figure 1: Left: microwave method. Right: routine method. Hematoxylin and eosin staining (×40)

Figure 2: Left: microwave method. Right: routine method. 0.1% Toluidine blue staining (×40)

Figure 3: Left: microwave method. Right: routine method. Periodic acid–Schiff staining (×20)

Figure 4: Left: microwave method. Right: routine method. Mucicarmine staining done (×40)
fixation of surgical and autopsied specimens. In the year 1985, Kok and Boon commenced microwave-assisted tissue processing. In routine histopathological procedure, the solutions gravitate to diffuse sluggishly into the tissue section, whereas this has been hastened by thermal conduction. This is the basis of microwave processing and staining. The mechanism of microwave processing is by instigating rotation of polar or charged molecules. This rotation principally acts on water molecule, which has mutually positively charged side and negatively charged side; therefore when the negatively charged sides are fetched close to an electromagnetic field, there is repulsion as they are like charges, triggering the molecules to rotate, this rotation consecutively happens through 180° at the rate of 2.45 billion cycles per second generating heat. 

Mayers showed that microwave-abetted tissue fixation has given the impression to advance the quality of fixation and to evade the loss of macroscopic details and shrinkage, which is by and large caused by routine fixation. Kennedy and Foulis testified that microscopic imaging of frozen section was substantively enhanced by using microwave-assisted fixation. Microwave-abetted tissue processing empowered faster processing of small to large tissue samples at much faster rate paralleled to that of routine processing. At the same time, the superiority of the microscopic image of the specimen was not conceded. The cytoplasmic and nuclear details were more substantial compared to the latter. A study conducted by Sivadas et al., where microwave-assisted fixation and processing was conducted in over 200 samples, showed enriched quality in microwave-assisted tissue processing related to that of conventional methods, which showed amplified shrinkage and incongruous fixation compared to the former. Our study also showed similar consistent results, wherein no statistically significant difference was observed between routine and microwave staining; in fact, the latter proved to replicate better cellular architecture compared to the former. The time so consumed for stains such as HE, TB, PAS, and MUC by conventional method was approximately 1 h 15 min, 55 min, 2 h, and 1 h, respectively, whereas by microwave-assisted staining, the time consumed fell down to 20, 17, 30, and 20 min, respectively. This enables faster visualization of tissue sections, leading to expeditious diagnosis. Similar results were obtained in studies conducted by Rohr et al., Kumar et al., Kennedy and Foulis, Patil et al., and Avwioro. Various studies, as brought up earlier, have been conducted to compare routine and microwave-assisted processing and staining but so far only one study has been reported on microwave staining using conventionally processed paraffin section. Our study was the subsequent one to report the significance of microwave staining of paraffin section, which was dealt using routine protocol, and the original study to report the significance of microwave-assisted MUC staining of mucocele sections. The results so obtained showed that the staining characteristics such as cell outline, cytoplasmic staining, nuclear outline, nuclear staining, nuclear chromatin, and staining intensity showed no significant difference as compared to routine staining method but the fact that time consumed by both these procedures showed a gargantuan difference, indoctrinates the importance of microwave staining in laboratory. Prompt diagnosis is an imperative part of treating each and every patient. Though various molecular techniques have been developed so far, the stint of histopathological reporting is an indispensable part of diagnostic pathology; use of microwave technology in the same is thus validated.

### Table 6: Chi-square test

| S. no | Stain                        | Cell outline clarity | Cytoplasmic stain | Nuclear outline | Nuclear staining | Nuclear chromatin | Staining intensity |
|-------|------------------------------|----------------------|-------------------|-----------------|------------------|-------------------|-------------------|
| 01    | Hematoxylin and eosin        | 0.53                 | 0.27              | 0.16            | 0.39             | 0.24              | 0.17              |
| 02    | Toluidine blue               | 1                    | 1                 | 0.65            | 0.65             | 0.55              | 0.37              |
| 03    | Periodic acid–Schiff (PAS)   | 0.53                 | 0.14              | 0.35            | 0.07             | 0.17              | 0.11              |
| 04    | Mucicarmine                  | 0.34                 | 0.34              | 0.49            | 0.49             | 1                 | 0.49              |

P value <0.05 is of significance

### Graph 1: Duration of routine and microwave staining
CONCLUSION

Microwave staining has been substantiated to play a significant role in diagnostic pathology in contemplation of analyzing further usage of the same; a larger sample size and different special stains might be used to further explore the role of microwave staining in oral pathology.

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Conflicts of interest
There are no conflicts of interest.

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