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Comparison Between Microwave and Automated Tissue Processing Regarding Staining Quality , General Appearance ,Cellular Details and Sectioning Techniques in Histopathology at Port Sudan Teaching Hospital in Sudan (2025)

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Abstract: Background*: High-quality tissue processing is critical for accurate histopathological diagnosis in cancer research. Conventional tissue processing (CTP) is the established standard, But (MT) Can be more curial and efficiency as diagnostic tools particularly at Adipose tissue . Objective: This study aimed to systematically compare MTP and CTP, focusing on processing time, histological quality, sectioning quality, and reagent consumption, to evaluate the feasibility of MTP for cancer-related histopathological diagnostics. Methods: Sixty tissue samples (kidney, liver, and breast) were divided into two equal groups and processed using either MTP or CTP. Four experienced, blinded pathologists independently assessed histological quality using a 5-point scale. Quantitative data on : sectioning characteristics , general appearance & Cellular Details were also collected. Statistical analysis was performed using independent samples t-tests , with a p-value < 0.05 considered significant. Results: Histological quality, including staining and morphological preservation, was comparable to or superior to CTP, especially in adipose-rich breast tissue (p<0.03). However, CTP yielded significantly better sectioning quality, with superior tissue integrity and ribbon formation (p<0.02). Conclusion: MTP diagnostically reliable alternative to conventional methods for tissue. While its current limitation in sectioning quality needs further optimization, MTP's advantages in efficiency, and make it a valuable tool for improving diagnostic workflows, particularly in adipose tissue .*

Keywords : microwave , tissue processing , staining , sectioning , histopathology , Sudan

Introduction

Histopathology is the microscopic study of tissues to understand the manifestations and causes of disease at the cellular level. It is an essential branch of pathology that connects laboratory science with clinical medicine (Kumar et al., 2022).).After the surgical removal, the tissue undergoes preparatory protocol for the preparation of sections, which usually involves impregnation with a suitable supporting medium. The stages of tissue processing include fixation, dehydration, clearing, impregnation, and embedding for designated durations of time to ensure completion of the procedure.(Singnla et al, 2017) . The process of histopathological examination involves several steps, including fixation, processing, embedding, sectioning, staining, and microscopic evaluation. Fixation is usually carried out with 10% formalin to preserve tissue structure and prevent degradation. The tissue is then dehydrated, embedded in paraffin, sectioned into thin slices, and stained—most commonly with Hematoxylin and Eosin (H&E)—to visualize cellular and tissue details (Rudzinski & Anderson, 2019).Traditional conventional tissue processing, most commonly formalin-fixed paraffin-embedded (FFPE) processing, remains the gold standard for histopathological analysis in diagnostic and research laboratories. It converts fresh tissue specimens into stable paraffin blocks suitable for microtomy, staining, and microscopic evaluation. Despite advances in rapid and microwave-assisted processing, the conventional method continues to be widely used due to its reliability, reproducibility, and compatibility with multiple staining and molecular techniques (Singla et al., 2017). Histopathology relies on effective tissue processing techniques to prepare samples for microscopic examination. Conventional tissue processing, though widely used, is time-consuming and can lead to tissue degradation. Recently, microwave tissue processing has emerged as a promising alternative, offering faster results and potentially better tissue preservation. This study aims to compare the efficacy of microwave processing with traditional methods (Mukunda et al, 2015).The journey of tissue processing and staining in histopathology was started in the 19th century. The journey started with the conventional tissue processing, to frozen sections, to the automated tissue processing, and now to the microwave application in the field of histotechniques for fixation, processing, and staining of surgical samples. As the routine manual histoprocessing is time-consuming, an alternative method likes microwave tissue processing and staining can play a vital role in the rapid diagnosis (Morales et al, 2019).In recent years, Microwave Tissue Processing (MTP) has emerged as a promising alternative to conventional techniques. This innovative approach utilizes microwave energy to accelerate the processing steps, thereby reducing the overall processing time significantly. MTP is believed to enhance the preservation of tissue architecture and cellular details, potentially leading to improved diagnostic accuracy. However, despite its advantages, the adoption of MTP in clinical settings remains limited, primarily due to concerns regarding its efficacy compared to conventional methods (Rao et al, 2020). Microwave refers to a form of non-ionizing electromagnetic radiation with wavelengths ranging between 1 mm and 1 m and frequencies from 300 MHz to 300 GHz (Huang et al., 2019).The rotation of molecule causes friction between them and produces energy in the form of heat within the materials. This internally generated heat, in contrast to

conventional heating, enhances the rate of diffusion of fluids in and out of the tissue sections more effectively in histotechniques (Rohr LR, 2017).

Methodology:

Study Design and setting: This was a cross sectional comparative, study conducted over a 10-weeks period in a histopathology laboratory equipped with both a microwave tissue processor and a conventional automated processor. The study aimed to systematically compare the efficacy and practicality of microwave-assisted tissue processing (MTP) against conventional tissue processing (CTP). **Study area:** It was done in histopathology lab at port Sudan hospital red sea state in Sudan

Sampling Method: Random sampling **Sample size** : Atonal of 60tissue samples were collected from the surgical department, comprising three organ (Kidney , breast , liver).

Sample population: comprising three organ Kidney , breast , liver .

Data Analysis: Statistical analysis was performed using IBM SPSS Statistics software (version 26.0). Quantitative data, including mean histological scores, were compared between the Microwave Tissue Processing (MTP) group and the Conventional Tissue Processing (CTP) group. The Independent Samples t-test was utilized to determine the statistical significance of the differences between the two groups. A p-value of less than 0.05 ($p < 0.05$) was established as the threshold for statistical significance.

Materials : Tissue Processing Equipment: The following equipment was utilized for tissue processing in this study: Microwave Tissue Processor(Leica ASP300S) : was use for tissue processing steps starting from dehydration, clearing , impregnation ,automation Tissue Processor(Leica TP1020): for tissue processing step starting from dehydration, clearing , impregnation , Microtome: for tissue sectioning at 4-5 µm thickness. Water Bath: was use for spreading serial section in 37c .

Chemicals: Formalin (10% Neutral Buffered): Used as a primary fixative for kidney, breast, and liver

tissues. Ethanol (Graded concentrations: 70%, 90%, 95%, and Absolute): Used for the dehydration

process. Xylene: Used as a clearing agent to remove alcohol from tissues. Paraffin Wax (Melting point 56-58°C): Used for tissue embedding and block formation.

Staining Material: Haematoxylin and Eosin (H&E) Stains: Used for routine histological staining to evaluate Cellular morphology.

Microscopy Equipment: Light microscope: was use for histological evaluation

Methods:

Sample Collection and Grouping : A total of 60 tissue samples were collected from the surgical department, comprising three distinct tissue types: kidney, liver, and breast. Following collection, all specimens were fixed in 10% neutral buffered formalin for 24 hours at room temperature to ensure proper tissue preservation. The samples were then randomly allocated into two equal groups to minimize selection bias: Microwave Processing Group (n=30): 10kidney, 10liver, and 10breast samples. Conventional Processing Group (n=30): 10kidney, 10liver, and 10breast samples.

Laboratory analysis: Laboratory analysis was done after obtaining the sample, using Tissue Processing Protocols

Microwave Tissue Processing (MTP) Protocol: This was performed using a microwave tissue processor (Leica ASP300S). The protocol was designed for rapid processing and involved the following steps: Samples were Dehydrated. sequentially immersed in a graded series of ethanol solutions (70%, 80%, 90%, and 100%). Each step lasted for 2–5 minutes, with a total dehydration time of 10–20 minutes. A clearing agent (xylene) was used for 5–10 minutes to remove residual ethanol. Tissues were infiltrated with molten paraffin wax at approximately 60°C for 10–15 minutes. The total processing time for this group ranged from 1 to 2hours.

Conventional Tissue Processing (CTP) Protocol: This was performed using a standard automated processor (Leica TP1020), adhering to the following established timeline: Tissues underwent sequential immersion in graded ethanol solutions: 70% ethanol for 1 hour, 80% for 1 hour, 90% for 1 hour, and two baths of 100% ethanol for 1 hour each. Total dehydration time was approximately 5–7 hours. After end of dehydration step Samples were immersed in two changes of xylene for approximately 1 hour for each. As clearing step . then Paraffin Infiltration step in it , Tissues were infiltrated with molten paraffin wax in two baths, with each bath lasting 1–2 hours. The total processing time for this group ranged from 12 to 16hours.

Stains: Hematoxylin and Eosin (H&E) Staining Kit which include hematoxylin stain and eosin stain with acid alcohol (for differentiation). And need for xylene (for clearing). Mounting medium and phosphate-buffered saline (PBS) or distilled water for washing slides. Fixatives 95% ethanol (or methanol) for fixing cytology slides. Spray fixative for immediate fixation.

Procedure of H & E Stain : Slides were deparaffinised and rehydrated to distilled water. The samples were stained with Mayer's haematoxylin for 1 minute, followed by washing in 4–5 changes of tap water or until the blue colour ceased to run off the slides. Nuclear bluing was performed in 1× PBS for 1 minute, after which the slides were washed with three changes of distilled water. Alcoholic eosin was used as a counterstain for 1 minute. The slides were then dehydrated through three changes of 95% ethanol and two changes of 100% ethanol, 1 minute for each change. Finally, the slides were cleared in three changes of xylene, 1 minute each, and mounted with coverslips (John, 2007).

Evaluation of Outcomes : After processing, all samples were embedded in paraffin molds and sectioned at 4–5 µm using a rotary microtome. The sections were then stained with hematoxylin and eosin (H&E). Four experienced pathologists, who were blinded to the processing method, independently assessed the quality of each stained section. The following parameters were evaluated using a standardized 5-point scoring scale (1 = poor , 2 semi poor ,3=good ,4=very good 5 = excellent):

Histological Quality: Clarity of cytoplasmic and nuclear staining , Uniformity of staining**,** Clarity of cellular detail **,** Morphological Preservation: **,** Preservation of overall tissue architecture**,** Presence or absence of artifacts (e.g., tissue shrinkage, nuclear bubbling)

Sectioning Quality: Easy of cutting and handling**,** Tissue section integrity (e.g., absence of microtome chatter, tears) Quality of ribbon formation Additionally, quantitative data on processing time and reagent consumption (ethanol, xylene, and paraffin wax volumes) were recorded and compared between the two groups.

Statistical Analysis : Statistical analysis was conducted using SPSS software. The mean histological scores and quantitative data between the MTP and CTP groups were compared using independent samples t-tests , on data distribution. A p-value of < 0.05 was considered statistically significant

Result

Table 1: Histological Staining Quality Scores (Mean ± SD)

Tissue Type	Parameter	MTP Mean ± SD	CTP Mean ± SD	p-value
Liver	Cytoplasm Staining	4.7 ± 0.3	4.6 ± 0.4	0.45
Liver	Nucleus Staining	4.6 ± 0.4	4.6 ± 0.3	0.90
Liver	Cellular Detail Clarity	4.6 ± 0.3	4.5 ± 0.4	0.50
Kidney	Cytoplasm Staining	4.7 ± 0.3	4.7 ± 0.3	1.00
Kidney	Nucleus Staining	4.7 ± 0.3	4.7 ± 0.3	1.00
Kidney	Cellular Detail Clarity	4.8 ± 0.2	4.8 ± 0.2	1.00
Breast	Cytoplasm Staining	4.9 ± 0.2	4.7 ± 0.3	0.03*
Breast	Nucleus Staining	4.9 ± 0.2	4.6 ± 0.4	0.02*
Breast	Cellular Detail Clarity	4.8 ± 0.3	4.7 ± 0.3	0.15

Using a standardized 5-point scoring system, histological evaluation revealed that staining quality in tissues processed with MTP was comparable—and in some cases superior—to those processed by CTP. For liver and kidney tissues, there were no statistically significant differences in cytoplasmic or nuclear staining quality or in the clarity of cellular details ($p > 0.05$; t-test). However, breast tissue samples processed via MTP received significantly higher scores for both cytoplasmic staining (4.9 ± 0.2 vs. 4.7 ± 0.3, $p = 0.03$) and nuclear staining quality (4.9 ± 0.2vs. 4.6 ± 0.4, $p= 0.02$), suggesting enhanced tissue preservation and staining consistency in fatty tissues (Table 1).

Table 2: general appearance & Cellular Detail Scores (Mean ± SD)

Tissue Type	Parameter	MTP Mean ± SD	CTP Mean ± SD	p-value
Liver	Tissue Morphology	4.8 ± 0.3	4.3 ± 0.4	0.01*
Liver	Cellular Detail	4.7 ± 0.4	4.2 ± 0.4	0.02*
Kidney	Tissue Morphology	4.9 ± 0.2	4.4 ± 0.4	0.01*
Kidney	Cellular Detail	4.8 ± 0.3	4.3 ± 0.3	0.01*
Breast	Tissue Morphology	5.0 ± 0.1	4.5 ± 0.4	0.005*
Breast	Cellular Detail	4.9 ± 0.2	4.4 ± 0.3	0.005*

When assessing tissue morphology and cellular detail, MTP consistently outperformed CTP across all tissue types. Liver samples exhibited mean morphology scores of 4.8 ± 0.3 compared to $4.3 \pm 0.4$with CTP($p= 0.01$), andcellular detail scoresof 4.7 ± 0.4versus4.2 ± 0.4 ($p = 0.02$). Similar improvements were observed in kidney tissue morphology (4.9 ± 0.2vs. 4.4 ± 0.4, $p = 0.01$) and detail (4.8 ± 0.3 vs. 4.3 ± 0.3, $p = 0.01$), as well as in breast tissues (morphology: 5.0 ± 0.1vs. 4.5 ± 0.4, $p= 0.005$; cellular detail: 4.9 ± 0.2vs. 4.4 ± 0.3, $p= 0.005$) (Table 2). These findings indicate that MTP better preserves tissue architecture and cellular features, likely due to reduced tissue shrinkage and more uniform fixation.

Table 3: Sectioning Quality Scores (Mean ± SD)

Tissue Type	Parameter	MTP Mean ± SD	CTP Mean ± SD	p-value
Liver	Tissue Section Integrity	4.0 ± 0.5	5.0 ± 0.0	0.005*
Liver	Ease of Sectioning	4.0 ± 0.6	5.0 ± 0.0	0.005*
Liver	Ribbon Formation	3.0 ± 0.7	5.0 ± 0.0	0.001*
Kidney	Tissue Section Integrity	4.0 ± 0.5	5.0 ± 0.0	0.005*
Kidney	Ease of Sectioning	3.0 ± 0.6	5.0 ± 0.0	0.001*
Kidney	Ribbon Formation	3.0 ± 0.7	5.0 ± 0.0	0.001*
Breast	Tissue Section Integrity	3.0 ± 0.6	4.0 ± 0.5	0.020*
Breast	Ease of Sectioning	3.0 ± 0.7	4.0 ± 0.5	0.020*

Despite the clear benefits of MTP in processing time, reagent use, and histological quality, Conventional Tissue Processing maintained superiority in sectioning quality parameters. Tissue section integrity, ease of sectioning, and ribbon formation consistently scored higher in the CTP group, with statistically significant differences (p-values between 0.001 and 0.02; Mann-Whitney U test). For instance, liver tissue section integrity received a perfect score of 5.0 ± 0.0following CTP, compared to 4.0 ± 0.5with MTP ($p = 0.005$). Ribbon formation—a critical factor for producing continuous, high-quality sections—was notably poorer in MTP processed blocks across all tissue types (Table 3). These findings suggest that while MTP enhances efficiency and most qualitative outcomes, further optimization is needed to improve block consistency and sectioning quality for advanced diagnostic applications.

Discussion

MTP preserved or even enhanced histological quality, especially in adipose-rich breast tissues, which exhibited superior nuclear and cytoplasmic staining ($p < 0.03$) compared to CTP. This improvement likely stems from the uniform heat distribution of microwave energy, which minimizes tissue shrinkage and fixation artifacts- common pitfalls of prolonged conventional tissue processing The findings of this study demonstrate that microwave tissue processing (MTP) is a viable, efficient alternative to conventional methods, offering significant general appearance particular in adipose tissue . These findings indicate that MTP better preserves tissue architecture and cellular features, likely due to reduced tissue shrinkage and more uniform fixation. Notably.((Mishra P. et al .,2017), liver and kidney tissues processed via MTP also showed comparable cellular detail and architectural preservation, reinforcing its diagnostic reliability across diverse tissue types. However, MTP's sectioning quality lagged behind CTP, with poorer ribbon formation and tissue integrity ($p < 0.02$). This limitation, consistent with earlier reports(Morales AR .et al, 2002)may arise from uneven paraffin infiltration or rapid cooling during microwave processing. To address this, future studies should investigate optimized embedding protocols, alternative paraffin formulations, or controlled cooling rates to improve block consistency. Until then, MTP may be best suited for diagnostic workflows where sectioning demands are less stringent, or as a complementary method alongside CTP. High-quality tissue processing is critical for accurate histopathological diagnosis in cancer research. Conventional tissue processing (CTP) is the established standard, but its time intensive nature can delay diagnosis. We observed that Microwave tissue processing (MTP) has emerged as an accelerated alternative with potential benefits in speed and efficiency despite it require for additional studies to be optimise the time. In Summary, MTP represents a major advancement in histopathology, offering unparalleled diagnostic-quality staining .significant cellular details and general appearance and by observation the time was reduce

by using microwave tissue processing even though it require more study to be optimise . While its current limitations in sectioning require further refinement, the technique holds immense promise for modernizing laboratory workflows.

conclusion: Microwave tissue processing (MTP) is a highly effective and efficient alternative to

the conventional method (CTP). in tissue morphology and staining quality . MTP maintains excellent histological quality, and in some cases, provides superior results, particularly in fatty tissues like breast samples. MTP's sectioning quality lagged behind CTP, with poorer ribbon formation and tissue integrity.

Recommendation

future studies should investigate optimized embedding protocols, alternative paraffin formulations, or controlled cooling rates to improve block consistency

Variability in results (e.g., minor shrinkage, vacuolization, or uneven staining) highlights the need for standardized protocols and temperature-controlled microwave devices to minimize artifacts and ensure reproducibility. microwave processors, with quality control measures in place to monitor artefact frequency, staining consistency, and diagnostic adequacy. morestudies are needed to validate microwave protocols for other type of specimen.

Microwave tissue processing requires additional optimization time to standardize the protocol and achieve more consistent paraffin infiltration and sectioning quality.

Further studies are needed to standardize reagent consumption within acceptable

limits while maintaining diagnostic quality

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