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ENHANCED THERMAL STRATERGY FOR RAPID ANTIGEN UNMASKING IN BREAST TISSUE FOR IMMUNOHISTOCHEMISTRY ANALYSIS.

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ABSTRACT

Introduction: Immunohistochemistry (IHC) is critical for breast cancer diagnosis, with antigen retrieval being a crucial step that affects diagnostic accuracy. Traditional pressure cooker methods suffer from variability and inefficiency, prompting the need for automated solutions.

Methods: This experimental study conducted at Saveetha Medical College compared a novel automatic thermal processor (ATP) with traditional pressure cooker methods for antigen retrieval in 50 breast cancer cases. The study evaluated estrogen receptor (ER), progesterone receptor (PR), and HER2neu marker detection efficiency, staining intensity, and diagnostic turnaround time between both methods.

Results: The ATP method demonstrated significantly higher detection rates for both ER (70% vs. 58%, p=0.031) and PR (70% vs. 50%, p=0.041) compared to the pressure cooker method. Staining intensity was significantly improved with the ATP method for both ER (p=0.035) and PR (p=0.036). The ATP also reduced diagnostic turnaround time by 28.6% (25 minutes vs. 35 minutes, p=0.001).

Discussion: The ATP provided superior antigen retrieval through precise temperature control, consistent heating, and automation, addressing the limitations of traditional methods. This innovation significantly improves diagnostic efficiency and accuracy in pathology workflows, particularly for hormone receptor assessment in breast cancer.

Keywords: Immunohistochemistry, Antigen retrieval, Automatic thermal processor, Pressure cooker, Breast cancer, Estrogen receptor, Progesterone receptor, HER2neu, Diagnostic efficiency

INTRODUCTION:

Immunohistochemistry (IHC) is an essential diagnostic tool in modern pathology that allows visualization of specific molecules within tissue using antigen-antibody reactions. Its importance has grown exponentially as more molecules involved in disease pathogenesis, diagnosis, and treatment are discovered. What makes IHC particularly valuable is its ability to preserve histologic architecture while assessing molecular expression patterns in their microenvironment [1]. A critical step in IHC is antigen retrieval, which enhances antigen visibility in formalin-fixed, paraffin-embedded (FFPE) tissue samples. During fixation, formaldehyde creates cross-links that mask epitopes—specific regions of antigens recognized by antibodies. This masking impairs antibody binding and affects staining results. Antigen retrieval methods reverse these cross-links, restoring antigenicity and improving detection sensitivity [2,3]. Traditional antigen retrieval has relied on pressure cookers, which, while effective, are labor-intensive, time-consuming, and prone to human error. This study introduces a novel automatic thermal processor (ATP) for rapid target antigen retrieval, representing a significant paradigm shift in immunohistochemical technique. The ATP utilizes preprogrammed temperature and timing parameters with specialized buffer solutions to optimize the retrieval process.[4-6]

The present study aimed to compare the superiority of automatic thermal processor with the pressure cooker method of antigen retrieval technique. Our specific objectives were to relate the precision of IHC staining by antigen retrieval between the automatic thermal processor and pressure cooker method; to analyze the intensity and proportion of ER, PR, and Her2Nu markers using both methods; and to evaluate the diagnostic turnaround time between the automatic thermal processor and pressure cooker method. The study particularly focused on breast cancer specimens, as accurate hormone receptor detection is crucial for treatment planning and prognostication. By comparing these two methods, we sought to determine whether the automated approach could improve standardization, reduce human error, and ultimately enhance diagnostic accuracy and efficiency.

MATERIALS AND METHODS :

This experimental study was conducted at the Department of Pathology, Saveetha Medical College, Chennai, after obtaining necessary approval from the Institutional Review Board (CTRI/2025/02/081495). The study period extended from January 2024 to May 2025, with a total duration of one year. A sample size of 50 breast cancer cases was included in the study, with inclusion criteria encompassing cases requiring immunohistochemistry for confirmation of histopathological diagnosis. Cases with inflammatory conditions were excluded from the study. The study was executed in two distinct phases. In the first phase, we developed the Automatic Thermal Processor (ATP), a novel device designed specifically for antigen retrieval. The ATP consisted of six key components: a heating chamber where tissue slides are placed; a temperature control system for precise regulation of heating; a timer to ensure consistent retrieval duration; reagent containers for buffer solutions; a circulating pump for temperature maintenance and efficient buffer mixing; and safety features to prevent overheating.

The ATP protocol utilized Citrate-Tris EDTA buffer (pH 9) at 110°C for 15 minutes, followed by washing with alkaline buffer Tris (pH 7.2), application of 3% hydrogen peroxide for 5 minutes, buffer wash, and IHC application (100 microliter per slide) with 1-hour incubation. Subsequent steps included buffer wash, binding reagent application for 15 minutes, Poly HRP application for 15 minutes, DAP application for 5-7 minutes, distilled water wash, haematoxylin counterstaining for 3 minutes, and final wash and mounting.

In the second phase, we conducted a comparative analysis of ER, PR, and HER2neu receptor staining proportion and intensity between the ATP and pressure cooker methods using identical tissue samples processed in parallel. Statistical analysis was performed using SPSS software version 27, employing Mann-Whitney U test for marker comparisons and unpaired t-test for turnaround time evaluation, with statistical significance set at $p<0.05$.

RESULTS:

The study population comprised 50 patients with breast cancer, with the majority falling in the 51-60 year age group (36%), followed by those above 60 years (28%), 41-50 years (24%), and less than 40 years (12%). Regarding diagnosis, invasive ductal carcinoma (NOS) was predominant, accounting for 94% of cases (n=47), while invasive lobular carcinoma, papillary carcinoma, and metaplastic carcinoma each represented 2% of cases (n=1 each).

For estrogen receptor (ER) assessment, the ATP method demonstrated significantly higher detection rates compared to the pressure cooker method, with 70% (n=35) positive cases versus 58% (n=29) in the pressure cooker method (p=0.031). Regarding ER staining intensity, the ATP method showed a reduction in negative cases (30% vs. 42%) and an increase in intermediate intensity cases (18% vs. 6%), while maintaining an equal proportion of strong intensity cases (52%) compared to the pressure cooker method (p=0.035).

TABLE 1: ESTROGEN RECEPTOR PROPORTION – COMPARISON

ER PROPORTION	PRESSURE COOKER	AUTOMATIC THERMAL PROCESSOR
POSITIVE	29	35
NEGATIVE	21	15
MANN WHITNEY U TEST		
P VALUE - 0.031		
SIGNIFICANT		

TABLE 2: IHC – ER – INTENSITY – COMPARISON

IHC- ER - INTENSITY	PRESSURE COOKER	AUTOMATIC THERMAL PROCESSOR
NEGATIVE	21	15
INTERMEDIATE	3	9

Similarly, for progesterone receptor (PR) assessment, the ATP method detected significantly more positive cases (70%, n=35) compared to the pressure cooker method (50%, n=25) with a p-value of 0.041. PR staining intensity also showed significant improvement with the ATP method, with fewer negative cases (20% vs. 44%), more intermediate cases (20% vs. 14%), and substantially more strong intensity cases (60% vs. 42%) compared to the pressure cooker method (p=0.036).

TABLE 3: PROGESTERONE RECEPTOR PROPORTION – COMPARISON

PR PROPORTION	PRESSURE COOKER	AUTOMATIC THERMAL PROCESSOR
POSITIVE	25	35
NEGATIVE	25	15
MANN WHITNEY U TEST		
P VALUE - 0.041		
SIGNIFICANT		

TABLE 4: IHC – PR INTENSITY – COMPARISON

IHC- PR - INTENSITY	PRESSURE COOKER	AUTOMATIC THERMAL PROCESSOR
NEGATIVE	22	10
INTERMEDIATE	7	10
STRONG	21	30
MANN WHITNEY U TEST		
P VALUE -0.036		
SIGNIFICANT		

For HER2neu evaluation, both methods identified an equal number of overall positive and negative cases; however, the distribution of scores differed significantly (p=0.019), with the ATP method showing more 2+ positive cases (30% vs. 12%) and fewer 3+ positive cases (10% vs. 32%) compared to the pressure cooker method.

TABLE 5: IHC – HER2 NEU COMPARISON

IHC- HER2NEU - ATP	PRESSURE COOKER	AUTOMATIC THERMAL PROCESSOR
NEGATIVE (0)	22	25
NEGATIVE (1+)	6	5
POSITIVE (2+)	6	15
POSITIVE (3+)	16	5
MANN WHITNEY U TEST		
P VALUE - 0.019		
SIGNIFICANT		

A key advantage of the ATP method was the significant reduction in turnaround time, with a mean time of 25 minutes compared to 35 minutes for the pressure cooker method (p=0.001), representing a 28.6% time saving.

DISCUSSION :

This study demonstrates that the Automatic Thermal Processor (ATP) method significantly improves the detection of hormone receptors in breast cancer specimens compared to traditional pressure cooker methods, with important implications for diagnostic accuracy and treatment planning. The ATP showed statistically significant increases in the detection of ER-positive cases (70% vs. 58%, p=0.031) and PR-positive cases (70% vs. 50%, p=0.041), highlighting the method's superior sensitivity in identifying clinically relevant markers. These findings are particularly significant in breast cancer diagnostics, where accurate hormone receptor status determination directly influences therapeutic decisions, including the application of hormonal therapies that can significantly impact patient outcomes. The enhanced detection rates with the ATP method might be attributed to its precise temperature control and consistent heating, which optimize the antigen retrieval process by effectively reversing formaldehyde-induced cross-links while minimizing tissue damage.[7,8]

Regarding staining intensity, the ATP method demonstrated notable improvements in detection quality for both ER and PR markers. For ER, the ATP detected more intermediate intensity cases (18% vs. 6%), suggesting better visualization of antigens that might be partially masked with conventional methods. For PR, the ATP identified significantly more strong intensity cases (60% vs. 42%), indicating improved antigen exposure and antibody binding, which enhances diagnostic confidence. These improvements in staining intensity have practical implications for pathologists, potentially reducing equivocal interpretations and minimizing the need for repeat testing. Our findings suggest that the ATP method may reduce the likelihood of false-negative results, which could prevent some patients from receiving potentially beneficial targeted therapies.

For HER2neu evaluation, an interesting pattern emerged wherein both methods identified an equal number of overall positive and negative cases, but with significantly different distribution patterns in scoring (p=0.019). The ATP method showed increased detection of 2+ cases (30% vs. 12%) and decreased detection of 3+ cases (10% vs. 32%) compared to the pressure cooker method. This shift in distribution warrants further investigation, as it may reflect either more accurate antigen retrieval with the ATP method or potentially different mechanisms of antigen exposure between the two techniques. Since 2+ HER2neu cases typically require additional confirmatory testing via fluorescence in situ hybridization (FISH), this finding has workflow and resource implications for diagnostic laboratories.[9-11]

A particularly compelling advantage of the ATP method was the significant reduction in turnaround time (25 minutes vs. 35 minutes, p=0.001), representing a 28.6% time saving. In busy diagnostic laboratories processing numerous specimens daily, this efficiency improvement could substantially impact workflow, potentially reducing reporting delays and improving laboratory productivity. The time efficiency, combined with the hands-free automated nature of the ATP, allows laboratory staff to focus on other tasks simultaneously, further enhancing overall laboratory

efficiency. This advantage becomes increasingly important as pathology laboratories face growing workloads and pressure to provide rapid results for timely clinical decision-making.[12]

Our findings align with previous research on antigen retrieval methods, though studies directly comparing automatic thermal processors with pressure cookers are limited in the literature. Research by Hammond et al. demonstrated better consistency in ER scoring with automated heat-induced epitope retrieval (HIER) systems across different laboratories [13], corroborating our finding that automated systems improve standardization. Similarly, Rhodes et al. emphasized the importance of standardized antigen retrieval protocols for reproducible results, highlighting that automated systems provide more precise control over retrieval conditions—a key feature of our ATP method. Kaur et al. [14] previously reported that pressure cooker methods offer advantages in terms of convenience and efficiency compared to microwave methods, but our study suggests that automated thermal processors further improve upon these advantages.

The ATP offers several key advantages over traditional methods that explain our findings. First, its precision in temperature control and consistent heating ensures optimal antigen retrieval conditions that maximize sensitivity while preventing overprocessing and tissue damage. Second, the ATP provides superior reproducibility through higher standardization with minimal operator-dependent variability, addressing a significant limitation of manual methods. This reproducibility is particularly valuable in clinical settings where consistent results across different operators and laboratories are essential for reliable diagnosis. Third, the ATP appears to better preserve tissue integrity with minimal damage, likely due to its precise control mechanisms that prevent excessive heat exposure. Finally, despite the longer total processing duration, the ATP significantly reduces hands-on time through automation, allowing laboratory personnel to engage in other productive tasks simultaneously.[15-17]

CONCLUSION:

The novel Automatic Thermal Processor for rapid target antigen retrieval represents a significant advancement in immunohistochemistry techniques with profound implications for clinical practice. Our study conclusively demonstrates that the ATP method provides superior detection of hormone receptors in breast cancer tissues compared to traditional pressure cooker methods, with improved staining quality, enhanced marker positivity rates, and significantly reduced turnaround times.

The standardization and reproducibility offered by the ATP address long-standing challenges in immunohistochemistry, particularly in the context of breast cancer diagnosis where accurate hormone receptor status determination directly influences treatment decisions and patient outcomes. While the pressure cooker method remains a viable and cost-effective option for lower-volume laboratories with budget constraints, the ATP's automated approach minimizes human intervention and variability, making it particularly valuable for high-throughput diagnostic laboratories where consistency and efficiency are paramount.

As immunohistochemistry continues to play an increasingly important role in cancer diagnosis and treatment planning, innovations like the ATP will help improve diagnostic accuracy, laboratory efficiency, and ultimately, patient care. Further studies with larger sample sizes across different tumor types would be valuable to fully explore the potential applications and benefits of this novel methodology across the broader spectrum of diagnostic pathology.

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