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A comparative analysis of two antigen retrieval techniques: Microwave oven and pressure cooker for immunoexpression of estrogen and progesterone receptors in breast cancer tissue

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ABSTRACT

Objectives: Antigen retrieval (AR) is an important step in Immunohistochemistry (IHC) which is used to unmask the antigenic sites and facilitate antigen-antibody binding. Adequate fixation of tissue is necessary to achieve consistent demonstration of tissue antigens that can be masked by the chemical process involved in formalin fixation and tissue processing. Out of the various methods of AR, heat-induced epitope retrieval (HIER) methods have greatly improved the quality and reproducibility of IHC. In this study, a comparison of the two most commonly used HIER methods-pressure cooker and microwave oven was done on thirty cases of breast carcinoma.

Materials and Methods: Appropriate tumor sections were taken and subjected to manual IHC testing for estrogen receptor (ER) and progesterone receptor (PR) receptors in each case. The results were divided into technique and microscopy-based. The parameters assessed on microscopy were uniformity of nuclear staining, quality of nuclear staining, internal control staining, presence of background staining, and Allred score. The sensitivity and specificity and positive and negative predictive values for each method were calculated.

Results: The parameters assessed on microscopy were comparable for both methods. Using a microwave oven, the sensitivity and specificity for ER and PR were 94% and 100%, respectively. Using a pressure cooker, the sensitivity, and specificity for ER were 94% and 100%, respectively, and for PR were 88% and 100%, respectively. On technical aspects, the pressure cooker method offers the advantage of being more convenient due to the possibility of simultaneous handling of more slides and being more time efficient.

Conclusion: Both the AR methods had comparable results on microscopy. However, the pressure cooker has the benefit of being both time and money efficient from a technical standpoint.

Keywords: Antigen retrieval methods, Estrogen receptor, Progesterone receptor, Microwave oven, Pressure cooker

INTRODUCTION

Immunohistochemistry (IHC) is used to characterize intracellular proteins or various cell surface receptors in tissues. Individual markers, or a panel of markers, can be used to characterize various tumor subtypes, confirm tissue of origin, distinguish metastases from primary tumors, and provide additional information regarding prognosis, predicting response to therapy or evaluating residual tumor post-treatment.[1] The analysis of estrogen receptor (ER) and progesterone receptor (PR) expression levels by IHC is an important part of the initial evaluation of breast cancer and is critically important in treatment planning.[2] There has been a gradual development of IHC methodologies, which have allowed the identification of specific and highly selective cellular epitopes, in formalin-fixed paraffin-embedded tissues with an antibody and appropriate labeling system.[3] Immunohistochemical methods ultimately depend on the good preservation of the specimen and of the target molecule. Long-term formalin tissue fixation results in antigen masking, probably through the aldehydic linkage between proteins and fixative molecules.[4,5]

Antigen retrieval (AR) refers to any technique in which the masking of an epitope is reversed and epitope antibody binding is restored. Heat-induced epitope retrieval (HIER), also known simply as AR, was pioneered in the early 1990s [6,7] after it had become apparent that the use of enzyme digestion alone to improve IHC staining was inadequate. As a general

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rule, all HIER procedures involve heating slide-mounted specimen material in a buffer solution, followed by a cooling-off period. Heat causes cross-linked protein epitopes to "unfold" (in a manner similar to deoxyribonucleic acid denaturation), while buffer solutions aid in maintaining the conformation of the unfolded protein.[8] However, HIER is better for unmasking epitopes because it is easier to use and produces better results compared to enzyme treatment.[9]

Although it is not possible to standardize AR methods by manual IHC methods after the evolution of automated IHC but this type of AR can be used in limited resource settings as it is cost-effective, does not require much space, and is flexible with regard to options of using different buffers and times at different temperatures.

There are limited numbers of centers offering immunohistochemical evaluation, and automated IHC may not be affordable by small-scale laboratories. In the present study, we sought to compare the two widely used heat-induced AR methods-microwave ovens and pressure cookers using household appliances to study the effect on ER and PR immunoexpression in breast cancer.

MATERIALS AND METHODS

This was a prospective study conducted on 30 breast resection specimens.

Inclusion criteria

- All the female patients with diagnosed breast carcinoma on fine needle aspiration cytology (FNAC) or Core biopsy irrespective of their grade, stage, and age who underwent treatment at Homi Bhabha Cancer Hospital, Sangrur (A Unit of TMC, Mumbai) were included in the study
- Adequately fixed resected specimens (Modified radical mastectomy (MRM)/Breast conservation surgery (BCS)/ Lumpectomy) were included in the study.

Exclusion criteria

- Review slides/Blocks with suboptimal processing were excluded from the study
- Post-neoadjuvant chemotherapy (NACT) cases were excluded from the study.

Diagnosed cases of breast carcinoma on FNAC or core biopsies irrespective of their grade, stage, and age were included in the study. Cases that had received NACT were excluded from the study. All specimens were adequately fixed, grossed, and processed as per standard protocol. The stained sections were examined for the presence of tumors. Appropriate tumor sections were then subjected to IHC testing for ER and PR receptors.

4 µm thick sections were cut for ER and PR IHC. Two sections were taken from each case on charged slides along with external positive control. Tissue sections were then dried for 10 min at room temperature and were subjected to overnight incubation at 37° C in the incubator. On the next day, the tissue sections were incubated at 65° for 30 min. Sections were deparaffinized with xylene and hydrated with graded alcohols. Sections were then treated with 3% methanol/ hydrogen peroxide solution for 30 min to prevent non-specific background staining. Tumor sections from each case were subjected to two different AR techniques. Slides were placed in a domestic microwave oven [Figure 1a and b] for AR in sodium citrate buffer solution (pH 6.0) in three cycles:

- First cycle (minimum heating): 5 min at 350W and then cooled at room temperature for 5 min
- Second cycle (moderate heating): 5 min at 500W and then cooled at room temperature for 5 min
- Third cycle (maximum heating): 5 min at 700W and then cooled at room temperature for 5 min.

The second set of slides was subjected to a domestic pressure cooker [Figure 1c and d] for AR in sodium citrate buffer (pH 6.0). First, the pressure cooker was preheated for 5 min. Afterward, a plastic rack holding slides and the appropriate amount of buffer solution was kept inside the pressure cooker and AR was done until the pressure cooker is fully pressurized (two whistles).

The slides were then rinsed in Tris wash buffer for 5 min. For immunostaining, sections were treated with 100 µL of primary ER antibody (SP1) and PR antibody (1E2) for 60 min and then rinsed with TRIS wash buffer for 5 min. The slides were then treated with 100 µL of standard horseradish peroxidase-labeled polymer for 30 min. In the end, Diaminobenzidine (DAB) substrate solution was applied on the tissue section for 5 min, rinsed in running tap water for 1–2 min, and counterstained with Harris hematoxylin for 10 s. Slides were then dehydrated in graded alcohols, cleared in xylene, and mounted in Dibutylpathalate Xylene (DPX).

Figure 1: (a and b) Microwave oven antigen retrieval method. (c and d) Pressure cooker antigen retrieval method.

The slides were then assessed on microscopy by the Pathologist and the immunoexpression of ER and PR Receptors was scored based on the "Allred score."[10] Expression of ER and PR in at least 1% of tumor cells was taken as positive and the Allred score was calculated in each case as per American society of clinical oncology (ASCO) and the college of American pathologists (CAP) guidelines[10]. The immunoexpression of the ER and PR receptors on the initial core biopsies/resection specimens of the cases which were subjected to automated IHC on Autoimmunostainer Ventana benchmark XT was taken as the reference. The following parameters were used for comparing the staining in both the AR methods: Uniformity of nuclear staining, nuclear staining quality, internal control staining, background staining, and Allred score. The sensitivity and specificity and positive and negative predictive values for each method were calculated.

RESULTS

The results were divided into technique-based and microscopy-based. Both techniques have their advantages and disadvantages which are outlined in Table 1. On microscopy, the quality and uniformity of nuclear staining and background staining, as well as Allred scores, were comparable in both methods [Table 2 and Figure 2a-d]. Uniformity of staining was found to be better in microwave oven AR as compared to pressure cooker AR. Sensitivity for ER IHC performed manually on microwave oven as well as on pressure cooker was 94% and specificity was 100%. Sensitivity and specificity for PR IHC performed manually on microwave oven were calculated and it was 94% and 100% and on pressure cooker and was 88% and 100%, respectively. The positive and negative predictive values were also calculated in both AR methods [Table 3].

There was no false positive case in both the methods, while there was one false negative case in both ER and PR using the microwave oven method. On using the pressure cooker method, one false negative case was found in ER while two false negative cases were found in PR. Comparison of the individual Allred scores of manual and automated IHC revealed similar results in most cases with both ER and PR. In three out of 30 cases, a higher Allred score was observed in manual IHC as compared to the automated IHC.

DISCUSSION

The analysis of ER and PR expression levels by IHC is an important part of the initial evaluation of breast cancer and helps in assisting treatment planning.[2]

The present study was undertaken on 30 cases of breast carcinoma to compare two methods of heat-induced AR -microwave oven and pressure cooker for manual IHC in the assessment of expression of ER and PR.

On comparing the expression of ER and PR in both methods, we found a similar rate of expression for ER in both methods (57%) while there was only a marginal increase in PR expression by microwave oven (53%) as compared to the pressure cooker (50%).

Table 1: Comparison of the advantages and disadvantages of microwave oven and pressure cooker antigen retrieval method.

Microwave oven		Pressure cooker	
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
1. Temperature is maintained properly.	1. Buffer level should be checked after some time as high temperature causes overflow of buffer.	1. Consumes less time.	1. Not possible to maintain pressure in domestic pressure cooker.
2. Consumes less space.	2. Time consuming.	2. Cost effective.	2. Requires an additional heating source such as Hot plate.
3. Easy to use and is automatic.	3. Cooling of slides is necessary after each cycle as providing constantly high temperature can cause detachment of tissue sections from slides.	3. No production of hot and cold spots.	3. Preheating is required.
4. No preheating is required.	4. Not possible to use steel slide racks.	4. Ability to use metal slide racks.	4.Not possible to monitor buffer level during retrieval process as pressure cooker lid is locked.

Table 2: Immunoexpression of ER and PR on automated, microwave oven, and pressure cooker IHC.

Parameter	Automated IHC (*n*=30)		Microwave oven (*n*=30)		Pressure cooker (*n*=30)	
	Positive staining *n* (%)	Negative staining *n* (%)	Positive staining *n* (%)	Negative staining *n* (%)	Positive staining *n* (%)	Negative staining *n* (%)
ER	18 (60)	12 (40)	17 (57)	13 (43)	17 (57)	13 (43)
PR	17 (57)	13 (43)	16 (53)	14 (47)	15 (50)	15 (50)

IHC: Immunohistochemistry, ER: Estrogen receptor, PR: Progesterone receptor

Table 3: Comparison of microwave oven AR and pressure cooker AR method taking automated IHC as gold standard.

Method	IHC	Total	Sensitivity (%)	Specificity (%)	PPV* (%)	NPV** (%)
Microwave oven	ER	30	94	100	100	92
	PR	30	94	100	100	93
Pressure cooker	ER	30	94	100	100	92
	PR	30	88	100	100	87

*PPV: Positive predictive value. **NPV: Negative predictive value, IHC: Immunohistochemistry, ER: Estrogen receptor, PR: Progesterone receptor, AR: Antigen retrieval

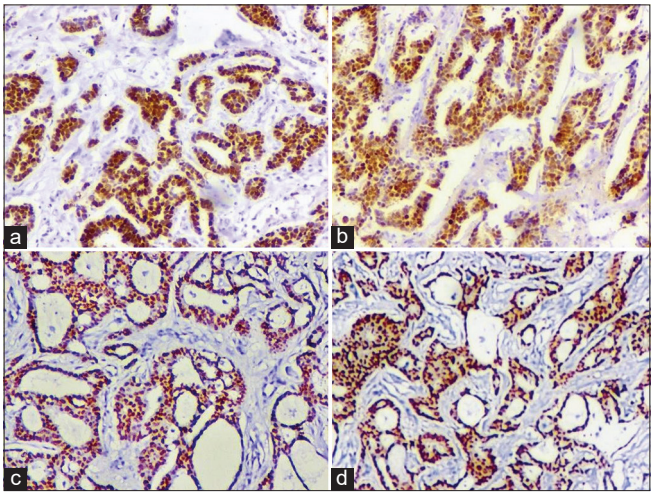

Figure 2: (a) ER immunoexpression by microwave oven method. (b) PR Immunoexpression by microwave oven method. (c) ER immunoexpression by pressure cooker method. (d) PR immunoexpression by pressure cooker method. ER: Estrogen receptor, PR: Progesterone receptor.

This contrasts with the findings of Qadir *et al.*[11] who demonstrated a higher ER expression with a pressure cooker as compared to microwave heating. They did not study PR expression in their study. Furthermore, the quality and uniformity of nuclear staining were comparable in both methods. While no false positive was found in either of the two methods, one false negative each was observed in both ER and PR using a microwave oven. On using the pressure cooker method, one false negative was found in ER while two false negatives were found in PR[12] (Neves *et al.*) also found no false-positive results in their study. They however found more false negatives with the microwave oven and no false negatives with the pressure cooker.

Out of the false negatives in PR expression, one occurred with the microwave oven method while two occurred with the pressure cooker method. The case which was a false negative on the microwave oven also turned out to be a false negative on the pressure cooker. For this case, even the ER expression was reduced with both the methods. The reference Allred score for this case was 7/8 in ER and 6/8 in PR. Manual IHC in two different tumor sections was performed but similar results were seen. One of the reasons for this false negativity for PR expression and reduction in ER expression could be attributed to improper fixation as the internal control was also not stained in this case. Improper fixation is an important cause of false negatives.[10]

Improper fixation might also be the most probable reason for the single false negative observed in ER expression in both microwave oven and pressure cooker methods which occured in the same case. The other false negative observed in PR expression occurred using the pressure cooker method. The reference Allred score for this case was 7/8. This case had strong ER expression using both microwave oven and pressure cooker. PR expression with microwave oven was also strong and the Allred score was 5/8. In this case, the false negative could be procedure-related.

Sensitivity and specificity were also calculated for ER and PR in both methods using the automated IHC Allred score as a reference. For expression of ER, we observed a sensitivity of 94% for both microwave oven and pressure cooker. The sensitivity for PR expression was also comparable between a microwave oven and pressure cooking (94% vs. 88%). On comparing the individual Allred scores of manual and automated IHC, almost similar Allred scores were observed in most cases in both ER and PR. In a few cases, a higher Allred score was observed in manual IHC as compared to the automated IHC.

CONCLUSION

With the advent of automated IHC platforms, the need for manual retrieval techniques is diminishing. However, in a developing country like India with many small-scale laboratories with limited resources, manual IHC is still cost-effective, does not require much space, and is very flexible with regards to experimenting with different buffers, time, and temperature.

In such circumstances, manual IHC with proper standardization can be utilized. Manual staining offers an advantage pertaining to flexibility in choosing reagents and retrieval methods and the possibility of applying subtle variations in technique when optimizing a staining protocol.[13] On technical aspects, a pressure cooker offers the advantage of being more convenient due to the possibility of simultaneous handling of more slides and being more cost-effective and less time-consuming.

The retrieval methods need to be decided keeping the needs of the laboratory in mind along with the cost factor. Following standard protocols at all steps would ensure optimum AR and in turn ensure accurate results which ultimately impacts patient management.

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Ethical approval

The research/study complied with the Helsinki Declaration of 1964.

Declaration of patient consent

Patient consent not required as patients identity is not disclosed or compromised.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

REFERENCES

1. Zaha DC. Significance of immunohistochemistry in breast cancer. World J Clin Oncol 2014;5:382-92.
2. Hicks D, Dell'Orto P, Falzon M, Hoff KD, Levy YY, McMahon L, *et al*. Immunohistochemical performance of estrogen and progesterone receptor antibodies on the Dako Omnis staining platform: Evaluation in multicenter studies. Appl Immunohistochem Mol Morphol 2017;25:313-9.
3. Suvarna KS, Layton C, Bancroft JD. Immunohistochemical and immunofluorescent tecniques. In: Bancroft's Theory and Practice of Histological Techniques. 8th ed. Netherlands: Elsevier; 2019. 346-47.
4. Shi SR, Cote RJ, Chaiwun B, Young LL, Shi Y, Hawes D, *et al*. Standardization of immunohistochemistry based on antigen retrieval technique for routine formalin-fixed tissue sections. Appl Immunohistochem 1998;6:89-96.
5. Miller RT, Swanson PE, Wick MR. Fixation and Epitope retrieval in diagnostic immunohistochemistry: A concise review with practical considerations. Appl Immunohistochem Mol Morphol 2000;8:228-35.
6. Shin RW, Iwaki T, Kitamoto T, Tateishi J. Hydrated autoclave pretreatment enhances tau immunoreactivity in formalin-fixed normal and Alzheimer's disease brain tissues. Lab Invest 1991;64:693-702.
7. Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. J Histochem Cytochem 1991;39:741-8.
8. Myers J. Overview of heat-induced epitope retrieval (HIER) techniques and devices. Available from: http://www.ihcworld.com/_protocols/epitope_retrieval/overview.htm [Last accessed 2016 Feb 24].
9. Kahveci Z, Minbay F, Noyan S, Çavusoglu I. A comparison of microwave heating and proteolytic pretreatment antigen retrieval techniques in formalin fixed, paraffin embedded tissues. Biotech Histochem 2003;78:119-28.
10. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, *et al*. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol 2010;28:2784-95.
11. Qadir A, Mushtaq S, Sharif MA, Mamoon N, Khadim MT. Comparison of heat induced antigen retrieval techniques for immune-histochemical estrogen receptor expression in ductal carcinoma of breast in females. J Coll Physicians Surg Pak 2010;20:519-23.
12. Neves JI, Begnami MD, Arias V, Santos GC. Antigen retrieval methods and estrogen receptor immunoexpression using 1D5 antibody: A comparative study. Int J Surg Pathol 2005;13:353-7.
13. Prichard JW. Overview of automated immunohistochemistry. Arch pathol Lab Med 2014;138:1578-82.

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