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Utility of manually constructed large core micro-array for routine immunohistochemical assessment of ER, PR and HER2 in carcinoma breast

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Abstract

Aim: To compare Immunohistochemistry (IHC) results of large core Tissue micro array (TMA) with the conventional whole section for estrogen receptor (ER), progesterone receptor (PR) and HER2 expression in breast carcinoma cases.

Methods: We analyzed 51 breast carcinoma cases. Large core TMAs were manually constructed with easily available materials. Immunohistochemical evaluation of ER, PR and HER2 were carried out in parallel by TMA and whole section methods for all cases as per Allred scoring system. Observed IHC scores were compared.

Results: We detected a high concordance rate of 98%, 100% and 98% for ER, PR and HER2 respectively between TMA and whole sections. In addition, the use of TMA caused a significant reduction in the use of laboratory resources- reagents, slides and blocks.

Conclusion: Our analysis showed various advantages of TMA over whole sections in IHC assessment. Hence, TMA can be implemented as a valuable tool in the routine assessment of breast biomarkers without compromising quality.

Keywords: Tissue microarray analysis (TMA), Immunohistochemistry (IHC), Biomarkers, Breast, Carcinoma

Introduction

Tissue micro array (TMA), first described by Kononen et al., is an analysis of multiple paraffin embedded tissues specimens simultaneously by combining thin cores of each sample in a single paraffin block. Up to hundreds of cores – of different pathological/normal tissues can be brought together for study in a single paraffin block.[(1)] Initially used for molecular profiling of tumor specimens, TMA has been used in the recent years for study of diagnostic, prognostic and predictive markers of breast cancer by immunohistochemistry (IHC).[(2,3)]

Anna Sapino et al. have used TMA with minimum four cores from different tumor foci for each case to demonstrate a good correlation between TMA and whole section IHC for breast biomarkers.[(4)] Use of TMA routinely for IHC study of breast biomarkers will drastically bring down high cost of reagents, save time, improve quality control process and increase overall efficiency of histopathology laboratory.[(5)] Prohibitive cost of commercially available array instruments prevents their use in an under resourced setting. Many modifications of array constructions including one without a recipient block have been described in the literature which overcome this problem.[(6,7,8,9)]

Aim of this study was to manually construct a simple, inexpensive microarray of large core diameter with easily available materials for routine assessment of breast biomarkers, thereby decreasing cost to a great extent and save time, resources and also increase efficiency. This was done keeping in mind the difficulties faced by a low volume, under resourced laboratory with low affordability of patients. And thus make such expensive, non-subsidised and now clinically mandatory testing for prognostic and predictive biomarkers of breast carcinoma affordable at a low cost. In this study we compare the results of large core TMA for Estrogen receptor, progesterone receptor and HER2 by IHC with that of whole sections.

Methodology

This is a prospective study of resected specimens of carcinoma breast received at Department of Pathology, JJM Medical College Davangere, Karnataka, India between December 2013 to April 2015 for ER, PR and HER2 testing. In all cases, conventional whole section IHC staining for ER, PR and HER 2 were done. Subsequently followed by staining on TMA sections from two to three cases accumulated at the end of 15 days.

For all cases of Carcinoma breast, samples were immediately fixed in 10% Neutral buffered formalin for a period of more than 6hrs up to maximum of 48hrs and care was taken to avoid overfixation.

Preparation of tissue core from donor block: Routine H&E on whole sections was done for all cases and were studied to confirm adequacy of the sample. Four micron thick sections were cut for IHC study on Poly L lysine coated slides for ER, PR and HER2 staining. Morphologically well preserved tumor foci (avoiding necrotic or sclerotic area) was selected and marked on H & E sections. Marked H&E slides were superimposed on the paraffin block and corresponding area of the tissue was marked for harvesting the core. Coring from the selected area was done to yield a large diameter core using a 3mm Skin Punch Biopsy Needle.

Preparation of Recipient Block: Dummy wax block without embedding any tissue was prepared. Using a

Punch Biopsy Needle of 3 mm diameter, desired number of holes were punched out in a grid like fashion.

Preparation of Tissue Array: Harvested tissue cores were arrayed into the recipient paraffin block. Number of breast carcinoma cases varied from two to three for each TMA block depending upon the workload. Positive control cores for ER/PR and HER2 and one orientation core were also included making up to five to six cores per TMA block. After array construction, recipient block was kept in the incubator at 50^0 C for 20 min for annealing, followed by cooling at 4^0 C for 5 min. Each TMA block was identified with a unique number and an array database was prepared in a worksheet for every array constructed.

IHC procedure: Four micron thick sections were cut from the TMA block and taken on Poly L lysine coated and on uncoated slides. Whole sections taken on coated slides from donor block and sections from TMA block were stained with FDA approved Anti Human ER (Clone 1D5), Anti Human PR (Clone PR88) and Anti Erb-B-2/Her 2 (Clone EP 1045) and BioGen X Super sensitive Polymer HRP detection system for visualization of primary antibodies after antigen retrieval by BioGenex EZ-Retriever system.

Reporting of results: ER and PR results were interpreted semi quantitatively according to Allred scoring system, which considers staining intensity of nuclei on a scale of 0-3 and proportion of positive cells scored on a scale of 0-5. Both these scores were then added and a score of 0 -2 was regarded as negative while 3 to 8 considered as positive.[10] HER2 IHC scoring was reported as negative (0/1+), equivocal (2+) and positive (3+) depending on intensity of cell membrane staining and whether or not >10% of tumor cells are stained.

Results

Total of 23 TMA blocks were created from 56 cases. It took 20 to 30 min to construct each block after acquiring some technical expertise. Difficulties encountered initially were block fracture - both donor and recipient, uneven levels of cores and dislodgement of tissue core while sectioning.

Some TMA sections suffered core loss while trimming/sectioning or during antigen retrieval process. Sections with core loss, either of a case or control were excluded from further analysis. Finally 51 cases with 21 TMA sections stained for ER, PR and HER2 were available for comparison with whole section IHC. The assessment of ER, PR and HER2 protein expression by IHC was performed in parallel on TMA and whole sections of the corresponding cases.

Out of 51 cases, 23(45.1%) cases and 22 (43.1%) cases were ER positive on whole sections and TMA respectively. While ER negative were 28(54.9%) cases and 29(56.8%) cases on whole sections and TMA, respectively (Table 1).

Table 1: Comparison of ER results on TMA with whole sections

	Negative	Positive	Total cases
Whole section	28(54.9%)	23(45.1%)	51
TMA	29(56.8%)	22(43.1%)	51

Concordance for ER staining was 98% between TMA and whole section with a sensitivity and specificity of 95.7% and 100% respectively. Only one case showed discordant result being reported as positive in whole section while negative on TMA. Difference in scoring of ER results was reported only in two cases which included the only discordant case mentioned above and one more case which was scored as Intermediate positive (score 6) on whole section and Rich positive (score 7) on TMA section (Table 2).

Table 2: Comparison of ER IHC scores on TMA with whole sections

TMA IHC Scoring	Whole section IHC Scoring					
	0-2 (Negative)	3 (Very poor)	4,5 (Poor)	6 (Intermediate)	7,8 (Rich)	
0-2 (Negative)	28	1	0	0	0	29
3 (Very poor)	0	3	0	0	0	4
4,5 (Poor)	0	0	3	0	0	3
6 (Intermediate)	0	0	0	5	0	5
7,8 (Rich)	0	0	0	1	10	11
	28	4	3	6	10	51

PR results showed 100% correlation between whole section and TMA section results with a sensitivity and specificity of 100% each. (Table 3) Scoring discordance was seen in only two cases resulting in score change from Poor(4,5+) to Very poor(3+) in one case and Rich (7,8+) to Intermediate (6+) in another case on TMA sections (Table 4). In both cases, score discordance did not affect the final result.(Table 4)

Table 3: Comparison of PR results on TMA with whole sections

	Negative	Positive	Total cases
Whole section	33(64.7%)	18(35.3%)	51
TMA	33(64.7%)	18(35.3%)	51

Table 4: Comparison of PR IHC scores on TMA with whole sections

TMA IHC Scoring	Whole section IHC Scoring					
	0-2 (Negative)	3 (Very poor)	4,5 (Poor)	6 (Intermediate)	7,8 (Rich)	Total
0-2 (Negative)	33	0	0	0	0	33
3 (Very poor)	0	3	1	0	0	4
4,5 (Poor)	0	0	3	0	0	3
6 (Intermediate)	0	0	0	3	1	4
7,8 (Rich)	0	0	0	0	7	7
Total	33	3	4	3	8	51

In the present study, all the 8 cases which showed HER2 positivity (15.7%) on whole sections were also positive on TMA sections with 98% concordance.(Table 5) Only case discordant on TMA was underscored in comparison to whole section, down grading the category from equivocal to negative.(Table 6)

Table 5: Comparison of HER2 results on TMA with whole sections

	Negative	Equivocal	Positive	Total
Whole section	41(80.4%)	2(3.9%)	8(15.7%)	51
TMA	40(78.4%)	3(5.9%)	8(15.7%)	51

Table 6: Comparison of HER2 IHC Score on TMA with whole sections

TMA IHC Scoring	Whole section IHC Scoring			
	0/1+ (Negative)	2+ (Equivocal)	3+ (Positive)	Total
0/1+ (Negative)	40	0	0	40
2+ (Equivocal)	1	2	0	3
3+ (Positive)	0	0	8	8
Total	41	2	8	51

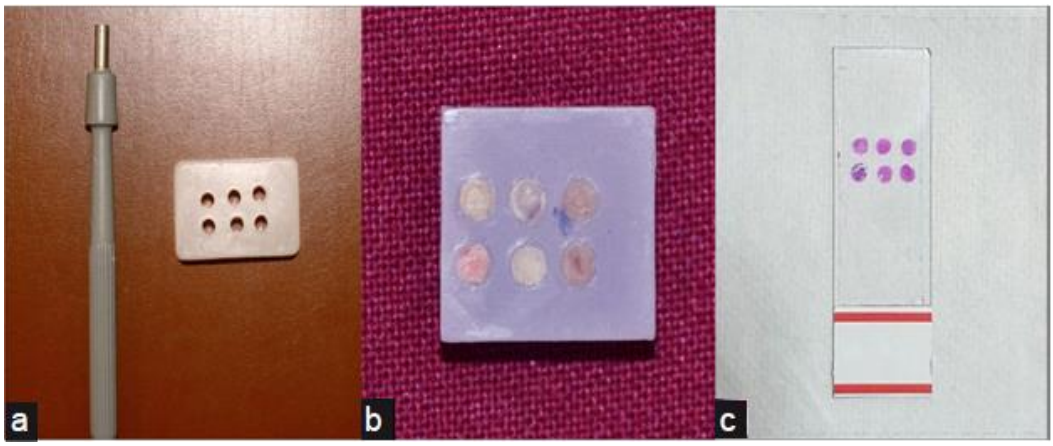

Fig. 1: a) 2mm skin punch biopsy needle with a recipient block. b)Prepared TMA block. c) Prepared TMA slide

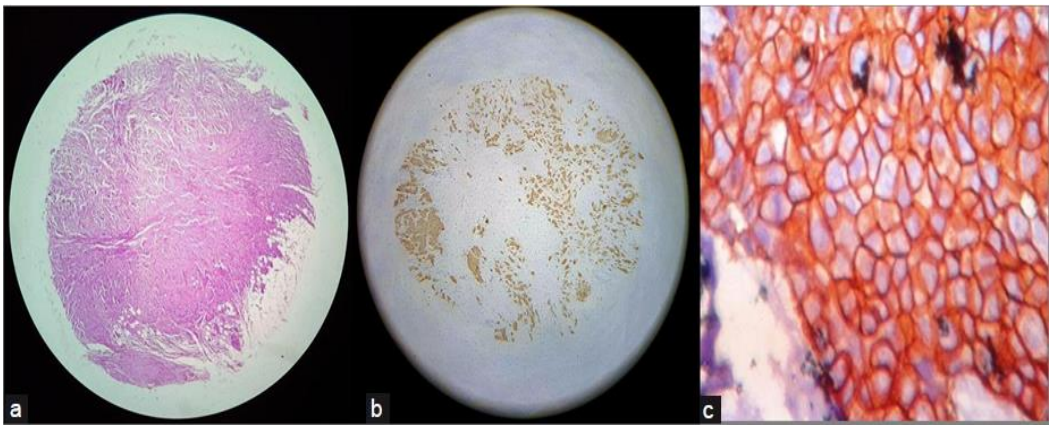

Fig. 2: a) H&E section, x40. b) Immunohistochemical staining for HER2 on paraffin embedded section, x40. C) HER2 staining, x400

Discussion

In the current situation, IHC is an integral part of Histopathology laboratory and prognostic/predictive biomarker testing has become clinically mandatory in breast carcinoma. It is a challenge for under-resourced and low volume laboratories situated in small cities serving people with low affordability to do IHC marker studies. We set out with the aim of bringing down the cost for ER, PR and HER 2 testing in our laboratory without compromising on quality. Singh et al., in their study have shown that IHC results on TMA sections have high concordance with whole section IHC and found that two core per case also gave very good concordance.[5]

In this study we compared TMA sections comprising one 3 mm core per case with whole sections of the corresponding case for breast biomarker analysis. All issues concerning quality maintenance were addressed – such as choice of fixative (10% Neutral buffered formalin), tissue anoxia time, minimum (6hr) and maximum (48hr) fixation time were considered for every case.[11]

In our experience TMA effectively decreased the technical and reagent demand on a per case basis. This also translates to decreased slide and block burden for archiving.(Table 7) These findings were similar to many previous studies.[3,5] Significant difference in processing time of cases on whole sections and TMA sections noted. More number of slides for whole section staining along with separate slides for positive and negative controls consumed more time & technical effort by manual staining method practiced in our lab. TMA sections not only had the advantage of decreased number of slides, but also more robust quality control as in built control cores & test cores go through exactly similar conditions.[5]

Table 7: Comparison of block/slide burden between whole section and TMA analysis

	Number of slides generated (including H&E)	Number of blocks generated
Whole section analysis	408	51
TMA section analysis	105	21

Laboratories with low volume of IHC will definitely save time, effort and money with TMA section IHC staining. In this study there was more than 50% decrease in overall cost burden with TMA section immunostaining compared to whole sections.

As quality control is a major challenge in IHC, all newer guidelines call for external quality assessment (EQA) program and proficiency testing to ensure strict quality management.[12,13] Also as HER 2 IHC poses greater challenges, FISH (Fluorescence in-situ Hybridization) study for over expression is advised in all equivocal results by IHC.[3] Non-availability of EQA/PT program for IHC and FISH study for HER2 were two major limiting factors in the study.

Tumor heterogeneity is also one important factor which influences analysis of IHC stains. Though careful selection of tissue, large diameter core used in our study might overcome this issue, we suggest all negative results to be confirmed on whole section if TMA sections are used for routine ER, PR and HER2 staining to rule out false negative results.

The results of our study indicates that the TMA based IHC analysis of breast biomarkers is a relatively cheap alternative to whole section analysis as it allows analysis of multiple tissue samples of different patients in an efficient and cost effective manner. It also showed comparable results with minimum use of expensive antibodies.

Conclusion

In the recent years, growing clinical demand of biomarker analysis in breast cancer has necessitated the need of prognostic & predictive biomarker assay by IHC in every histopathology laboratory. Thus TMA with its proven benefits can be an effective and cheaper alternative to the conventional whole section in IHC analysis in any laboratory with basic facilities and minimum expertise without compromising on quality. Therefore, TMA can be used more efficiently in the routine assessment of breast biomarkers and its implementation will definitely increases the overall laboratory performance.

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