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Cost Effectiveness of Manual Tissue Microarray Technique in Diagnostic Immunohistochemistry

Manoranjani Rajendran [1] , Nithya Ilanchezhian[2], Arasi Rajesh[3]
[1](Department of Pathology, Government Villupuram Medical College, India)
[2](Department of Pathology, Government Villupuram Medical College, India)
[3](Department of Pathology, Tirunelveli Medical College, India)
Corresponding Author : Nithya Ilanchezhian

Abstract:*Breast carcinoma is graded by Modified BloomRichardson score and predictive immunohistochemical markers are estrogen receptors, progesterone receptors and HER2/neu status. Conventionally these analyses are done by immunohistochemistry(IHC) on whole sections. In our study we use tissue microarray technique(TMA) where small representative tissue samples from many cases were assembled on a single histology slide and subjected to Immunohistochemical analysis. We assessed the cost-effectiveness of IHC done on TMA slides.*

50 cases of invasive breast carcinoma were included in the study. First the design for TMA construction was laid out. Paraffin embedded tissue blocks were collected and the areas of invasive carcinoma were cored from donor blocks and transferred to the recipient blocks using bone marrow needle. Thus tissue microarray was constructed manually. Immunohistochemical analysis using ER, PR and Her2/neu were done for all these cases.

Of the 50 patients analysed, majority were invasive ductal carcinoma (84%). Majority of the invasive breast carcinoma were of MBR grade II (50%) followed by grade III tumors (42%) and grade I tumors (8%). Among 50 cases, ER and PR were positive in 24 cases (48%) and 31 cases (62%) respectively. HER-2/neu expression was seen in 25 cases (50%). A statistically significant correlation was noted between histologic grading and ER, PR and HER2/neu status. The tissue microarray uses only one seventh of the reagent consumed by conventional immunohistochemistry.

The process of immunohistochemistry using manualtissue microarray obviates the need for control and standardisation. This allows the study of different cases on a single slide, thus reducing the amount of reagent, duration and labour of the procedure and making it cost effective.

Keywords: *Breast carcinoma, Immunohistochemistry, Manual Tissue microarray.*

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I. Introduction

Breast carcinoma is the most common malignant tumour and the leading cause of cancer deaths in women. In India the incidence of breast cancer is rapidly rising, amounting to 25%-33% of all cancers in women [1]. Breast carcinomas are a heterogeneous group of tumours with diverse behaviour, outcome and response to therapeutic agents [2]. Prognostic significance of breast carcinoma is no longer based on morphology alone. Modern day approach to cancer treatment involves identification of specific biomarkers on the tumour cells against which targeted therapy can be directed. ER, PR and Her2/neu markers are routinely evaluated in breast carcinoma. ER positive tumours are treated with anti-estrogen therapy (tamoxifan) and Her2 monoclonal antibody (trastuzumab) is effective in Her2 positive cases [3]. These markers are routinely evaluated by Immunohistochemistry (IHC) on whole sections.

While traditional techniques require processing and staining of several slides manually, TMA allows study of several cases by staining a single master slide. It saves time and cost of reagent used. It has the added advantage that all sections are processed at one time using identical conditions [4]. One of the major concerns regarding TMA is the small core size of tumour tissue and whether it is representative of the whole tumour. This study was hence conducted to determine the immunohistochemical reagents consumed in arrayed slide is less when compare to conventional methods in whole sections.

II. Aim

To assess cost effectiveness of Manual Tissue Microarray technique in diagnostic Immunohistochemical analysis

III. Materials And Methods

The study was conducted after obtaining approval from Institutional Ethical Committee . The study was carried out in theDepartment of Pathology, Tirunelveli Medical College and Hospital, Tirunelveli from January 2014 to September 2015

3.1 SOURCE

Formalin fixed, paraffin embedded tissue blocks from 50 surgically resected breast tissues which were diagnosed as invasive breast carcinoma by histopathological examination were retrieved along with their haematoxylin and eosin stained slides and they were examined and the tumor was graded according to Modified Bloom and Richardson grading system.Receptor status were studied for these 50 cases by conventional IHC methods.

The method for construction of manual tissue microarray includes the following steps.

1. Designing the layout for TMA construction.
2. Collection of the donor blocks.
3. Preparation of the recipient paraffin blocks.
4. Immunohistochemistry and analysis.

3.2 DESIGNING THE LAY OUT

Before constructing the array proper, the layout of the tissue microarray defining the geometric position of each tissue core in the recipient block was made. The grid was constructed in such a way that there was a single core from each case on the recipient block. The grid had blank cores in between the cores from the cases which helped in determining the position of the cases on the immunohistochemistry performed slides.

3.3 COLLECTION OF THE DONOR BLOCKS

The hematoxylin and eosin stained sections which were prepared from formalin fixed paraffin embedded blocks of all the cases of invasive breast carcinoma in the Department of pathology during the study period were retrieved. The corresponding formalin fixed paraffin embedded tissues were also obtained which constituted the donor block. Then the hematoxylin and eosin stained slides which contained full sections were examined and the area of interest was marked by using black glass marking pen. The area of interest is the area of tumor containing well preserved and well stained malignant cells. Then these marked areas on the slides were matched with the donor blocks and the corresponding areas over the donor blocks were also marked with the help of black glass marking pen. This area was used as the site for obtaining cores for the recipient block.

3.4 PREPARATION OF THE RECIPIENT PARAFFIN BLOCKS

The empty paraffin recipient blocks with minimum size of 25mm x 25mm were first prepared by freshly poured molten wax in the metal moulds. Then it was allowed to cool. Later using 16 gauge needle, paraffin wax cylinders of 2mm diameter were punched from the recipient blocks. Each block contained 3x3 cylinder matrix at a distance less than 2mm. In our study seven such blocks containing 50 cases were prepared.

Then using 14gauge bone marrow aspiration needle, tissue cylinders were obtained from the area of interest which were previously marked over the the donor blocks, after which it was injected into the recipient blocks into the corresponding empty cylinders with the help of predesigned layouts so that six recipient blocks contained seven cases and one recipient block contains eight cases. After the recipient block was embedded with the tissue cores, the block was incubated at 40^0c for 15 minutes and then it was allowed to cool for few minutes at room temperature and then the array was chilled on the ice for few minutes.

Fig 1. Completed manualtissue microarray block

3.5 IMMUNOHISTOCHEMISTRY

3.5.1 SECTION CUTTING

Sections were taken at 5 micron thickness after tissue microarray construction on the surface of the Poly-L-Lysine coated slides. This was followed by incubation of slides at 58-60^{0}c for one hour.

3.5.2ANTIGEN RETRIEVAL SOLUTION

We used two antigen retrieval solution and a wash buffer as prescribed by the manufacturer (PATH IN SITU).

1. Citrate buffer at a pH of 6.9 for HER 2 neu.
2. Tris EDTA at a pHof 9 for ER, PR.
3. Tris wash buffer at pHof 7.6 for both.

3.5.3 ANTIGEN RETRIEVAL

In our institution we followed antigen retrieval by using pressure cooker as it produces even heating with lesser disadvantages compared to other methods.

3.5.4 PROCEDURE FOR IMMUNOHISTOCHEMISTRY

Section cutting and incubation is followed by Xylene wash (2 changes) for 10 minutes each.

1. Rehydrated in graded alchohol containing 100%, 80%, 70% for ten minutes each.
2. Rinsed in distilled water for 2 minutes.
3. Antigen retrieval.
4. Cooling for 15 minutes.
5. Washed in TRIS wash buffer- 2 changes 5 minutes each.
6. Treated with peroxide block for 5 minutes.
7. Washed in TRIS wash buffer- 2 changes 10 minutes each.
8. Kept in protein block for 10 minutes.
9. Application of primary antibody (ER, PR, HER 2 neu) – 30 minutes.
10. Washed in TRIS wash buffer- 2 changes 10 minutes each.
11. Amplifier application for 15 minutes.
12. Washed in TRIS wash buffer- 2 changes 10 minutes each.
13. Application of secondary antibody (HRP POLYMERASE) – 20 minutes.
14. Washed in TRIS wash buffer- 2 changes 10 minutes each.
15. Application of Diamino-benzidine tetrachloride (DAB) chromogen 2 - 4 minutes.
16. Washed in distilled water – 2 changes.
17. Counterstaining was done with Hematoxylin for 30 seconds to impart background staining.
18. Wash in running tap water.
19. This is followed by dehydration, clearing and mounting.

3.5.5IMMUNOHISTOCHEMICAL EVALUATION

Immuohistochemical analysis of a panel of HER2/neu, ER, PR were done in paraffin embedded tissue samples using polymer HRP system based on non-biotin polymeric technology. 5 µ thick sections from formalin fixed paraffin embedded tissue samples were transferred onto Poly-L-Lysine coated slides. Heat induced antigen retrieval was done. The antigen was bound with rabbit monoclonal antibody against HER2/neu, ER, PR and then detected by the addition of secondary antibody conjugated with horse radish peroxidase - polymer and diaminobenzidine substrate. ER and PR scoring was done using quick scoring and Her2/neu scoring was done as per guidelines of CAP/ASCO [5,6].

Fig. 2 (A) - IHC slide of PR frommanual tissue microarrayed block

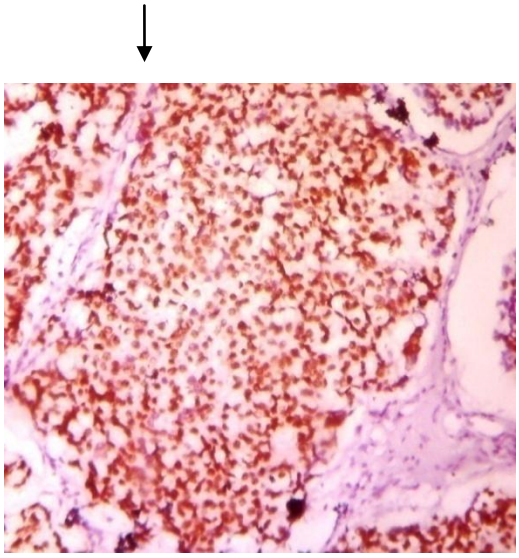

Fig. 2 (B) – Grade II IDC (NOS) showing intense PR (+) of score 8 in the above tumor (IHC, x100)

Fig. 3(A) - IHC slide of HER – 2 neu from manualtissue microarrayed block

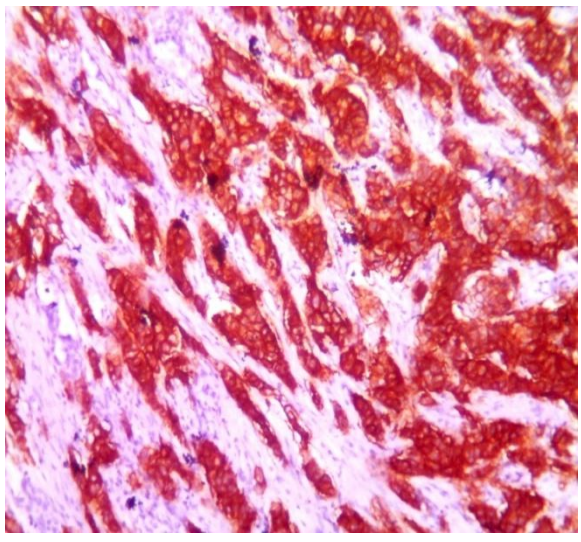

Fig. 3(B) – Grade III IDC (NOS) showing strong HER 2 (+) of score 3 in the above tumor (IHC, x100)

IV. Observation And Analysis

Of the 50 patients analysed 42 (84%) were IDC, NOS. Invasive lobular carcinoma constituted 6% of the group followed by papillary and mucinous carcinoma each constitutes 4%. Metaplastic carcinoma was the

least common type constituting 2% of the cases. Among these cases, majority of the patients 25 (50%) were of MBR grade II followed 21 cases were grade III (42%) and 4 cases (8%) were under grade I.

There was an almost equal distribution of estrogen receptor positive (48%) and negative cases (50%). 2% of sample was lost during tissue processing. While analyzing the progesterone receptor status, majority of tumors were receptor positive (62%). Only 32% of cases did not express progesterone receptor and remaining 6% of samples were lost during tissue processing. 50% of the cases demonstrated HER-2/neu positivity and 40% of cases were negative. 10% of samples were lost during tissue processing.There was a significant statistical association between the estrogen receptor, progesterone receptor and HER 2/neu status and the histological grade (p value: 0.008, Chi square test).

TABLE 1. RELATIONSHIP OF ER TO MODIFIED BLOOM RICHARDSON GRADE

MBR GRADE	ER(+)	ER(-)	P value* (Chi square test)
I	3	1	0.009
II	16	8	
III	5	16	

TABLE 2. RELATIONSHIP OF PR TO MODIFIED BLOOM RICHARDSON GRADE

GRADE	PR (+)	PR(-)	P value* (Chi square test)
I	4	0	0.008
II	18	4	
III	9	12	

TABLE 3: CORRELATION BETWEEN HER 2/neu TO MODIFIED BLOOM RICHARDSON GRADE

GRADE	HER 2/neu (+)	HER 2/neu(-)	P value* (Chi square test)
I	0	4	0.04
II	14	7	
III	12	9	

TABLE 4: QUANTIFICATION OF IHC REAGENTS USED IN CONVENTIONAL VS TMA SECTIONS

IMMUNOHISTOCHEMICAL REAGENT	TMA	CONVENTIONAL	CONSUMPTION RATIO
Primary antibody	0.11 IU	0.8 IU	1:7
Secondary antibody	0.11 IU	0.8 IU	1:7
Chromogen	0.11 IU	0.8 IU	1:7

The conventional immunohistochemistry using full section consumes 0.8 IU of the chemical reagents, whereas only one seventh of the reagent is consumed by manual tissue microarray.

Array sections from these 7 blocks were obtained which were subjected to immunohistochemical analysis showed 10.66% tissue loss.

V. Discussion

The present study included 50 cases of histologically diagnosed Invasive breast carcinoma and Modified Bloom and Richardson grading system was applied for all these cases. Tissue Microarray was constructed for all these 50 cases on seven blocks and they were subjected to Immunohistochemical analysis to study ER, PR and HER 2 status in these tumors.

In the present study out of 50 cases, 42 were IDC (NOS) which constituted 84% and 3 were ILC which constituted 6% which is comparable to the previous studies

In the studies conducted by Zafrani et al,[7] Onitilo AA et al [8] and Piero G et al,[9] the most common tumors were grade II which constituted 40%, 38.4% and 37% respectively. In present study also majority were grade II tumors which constituted 50% of the cases.

In the study done by Ayadi et al [10], 72.2% of ER positive cases were grade I and II whereas 22.5% of ER (+) cases were grade III tumors. In the present study, out of 50 cases, 65.51% of ER positive cases were grade I and II, whereas 23.8% of ER (+) cases were grade III tumors. In the present study the relationship between ER expression and histological grading was statistically significant (p value: 0.009). In Ayadi et al[10] study, 61.4% of PR (+) cases were grade I and II tumors and 27.5% of PR (+) cases were grade III tumors. In the present study, 75.86% cases were grade I and II and 42.85% cases were grade III tumors (Table.23). In the present study, the relationship between PR expression and histological grading was statistically significant (p value: 0.008).

In the study done by Ayadi et al[10], 14.8% of HER 2 (+) cases were grade I and II tumors and 27.5% cases of HER 2 cases were grade III tumors whereas in the present study 48.27% of HER 2 (+) cases were in grade I and II and 57.14% cases of HER 2 (+) were in grade III (Table.23). In the present study the relationship between ER expression and histological grading was statistically significant (p value: 0.04).

TABLE 5: COMPARISON OF STUDIES BASED ON ER, PR & HER 2 AND HISTOLOGICAL GRADES

	Ayadi et al[10]			Present study		
Histological grade	ER (+)	PR (+)	HER 2(+)	ER(+)	PR(+)	HER2(+)
Grade I and II	72.2%	61.4%	14.8%	65.1%	75.86%	48.27%
Grade III	22.5%	27.5%	27.5%	23.8%	42.85%	57.14%

According to Richter J et al [11], tissue loss due to technical problem ranged between 15-33%. D H Zhang et al[12], in his studyobserved tissue loss of 4% whereas in the present study overall tissue loss accounted to 10.66%.

To analyse single conventional tissue section immunohistochemically, minimum of two drops (0.8 IU) of chemical reagents which includes primary antibody, secondary antibody and DAB chromogen are required. Hence, a single tissue section when used conventionally consumes 0.8 IU of the reagent whereas in TMA, the same quantity has been used to analyze seven cores taken from seven different cases. So TMA, apart from having the advantage of parallel analysis of multiple sections, also decreases the time taken for the IHC procedure and the amount of chemical reagents used[9]. In this way, the use of immunohistochemical analysis in Invasive breast carcinoma by tissue microarray is helpful for economical and rapid study of receptor status.

VI. Conclusion

The process of immunohistochemistry using conventional tissue section consumes more reagents, also requires control and standardisation for each batch which is not needed while using tissue microarray. Immunohistochemical analysis with a panel of markers using tissue microarray also reduces time and labour. By taking representative cores from different cases and performing IHC on them on a single slide proved to be economical. Tissue loss due to technical problems can be overcome by following standard protocols or by obtaining more number of tissue cores.

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