

## RESEARCH PAPER

# Comparative study of various scoring systems (All red and H scoring) for evaluation of hormone receptor ( Estrogen and progesterone receptor) in breast carcinoma by immunohistochemistry.

Dr Nitanshi Gupta<sup>1\*</sup>, Dr Hari Shankar Pandey<sup>2</sup>, Dr Priyamvadasinghal<sup>3</sup>

<sup>1</sup>MD Pathology from Government Medical college, Dr sushila tiwari Government Hospital, Haldwani, Uttarakhand  
nitanshi.gupta22@gmail.com

<sup>2</sup>MD Pathology( Professor and HOD) in Government Medical college Haldwani, Uttarakhand  
Email ID:physianin@gmail.com

<sup>3</sup>MD pathology Associate Professor in department of Pathology of Government medical college Haldwani, Uttarakhand  
Email id:priyamvadasinghal15@gmail.com

### Abstract:

**Background:** Breast carcinoma is the commonly diagnosed cancer in females and express various receptors which are considered both prognostic as well as predictive markers. Immunohistochemistry is used to assess hormone receptor status evaluating hormone receptors Estrogen and Progesterone (ER/PR) in infiltrating ductal carcinoma of breast. Present study was undertaken to evaluate histomorphological feature of carcinoma of breast and grading by Modified Bloom Richardson Grading system, the status of hormone receptor by Allred scoring system and H scoring system along with correlation of Allred and H scoring system with Modified Bloom Richardson grading system and also to compare Allred and H scoring system.

**Methodology:** It was an institutional based observational study with cross sectional design and was conducted in the Department of Pathology and Department of Surgery of Government Medical College and Dr. Sushila Tiwari Government Hospital, Haldwani, Uttarakhand, India for 2 years. Study was conducted among 60 females study subjects. Data was collected after the informed consent of patients. Diagnosis of Breast carcinoma was made after Gross and Histopathological examination. Grading was done on Haematoxylin and Eosin stained slides by Modified Bloom-Richardson grading system followed by Immunohistochemical technique to study ER and PR receptors. Allred and H scores were calculated in terms of ER and PR. Kappa statistics was used to observe the agreement between Allred and H scoring system and p value was calculated to know the correlation of Allred and H score with Modified Bloom-Richardson grading system. p value less than 0.05 was considered as statistically significant.

**Results:** Total 60 Patients were included in the study. The common grade was grade II ((58.3 %) followed by Grade III (28.3%) and Grade I (13.3%). Maximum cases are ER(-)/PR(-) Negative in terms of Allred score and H score i.e. (50%) and (56.7%) respectively. Kappa statistics shows almost perfect agreement (k=0.833) between the Allred and H score in terms of detection of ER, however fair agreement (k=0.263) in terms of detection of PR

**Conclusions:** In our study quantification of ER/PR expression by both Allred score and H score methods produced comparable results and show high level of agreement between them. Both scores had shown statistically significant correlation with NMBR Grading system suggesting that both the scoring system can be used for the assessment of hormone receptor status.

**Key-words:** Breast carcinoma, Allred score, H scoring system, Hormone receptors

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### INTRODUCTION

Breast carcinoma is the most common malignancy occurring in females worldwide while in India it is the second most common malignancy occurring after cervical cancer in females.<sup>1</sup>

Breast carcinoma is a highly heterogeneous neoplasm with distinct subtypes. These subtypes are commonly grouped into four categories based on the immunohistochemical expression of hormone receptors: estrogen receptor positive (ER+), progesterone receptor positive (PR+), human epidermal growth factor receptor positive (HER2+), and triple-negative (TNBC), which is characterized by the lack of expression of any of the above receptors.<sup>2</sup>

Estrogen receptor (ER) is an important diagnostic determinant, as approximately 70–75% of invasive

\*Author for Correspondence: nitanshi.gupta22@gmail.com

breast carcinomas are characterized by significantly high ER expression.<sup>3,4</sup> The progesterone receptor (PR) is expressed in more than 50% of ER-positive patients, and very rarely in those with ER-negative breast cancer. PR expression is regulated by ER<sup>5</sup> therefore, physiological PR values inform about the functional ER pathway. However, both ER and PR are abundantly expressed in breast cancer cells, and both are considered diagnostic and prognostic biomarkers of breast cancer.<sup>6</sup> Immunohistochemical evaluations of hormone receptors (ER and PR) are based on two main features that are the proportion of stained cells and the intensity of the staining. Hence, for evaluating these two features, scoring systems such as Allred (quick) scoring system and histochemical (H) scoring system have been practiced at different centers.<sup>7,8</sup>

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Present study was undertaken to evaluate histomorphological feature of carcinoma of breast and grading by Modified Bloom Richardson Grading system, the status of hormone receptor by Allred scoring system and H scoring system along with correlation of Allred and H scoring system with Modified Bloom Richardson grading system and also to compare with each other.

**MATERIALS AND METHOD**

This institutional based observational study with cross sectional design was conducted in the Department of Pathology and Department of Surgery for 2 years in Department of Pathology and Department of Surgery of Government Medical College and Dr. Sushila Tiwari Government Hospital, Haldwani, Uttarakhand, India. Total 60 patients were studied who fulfilled inclusion criteria i.e.Women of all age group coming to surgery department and had undergone simple mastectomy, Modified Radical mastectomy, lumpectomy, Trucut biopsy for suspected malignancy and patients willing to participate in this study. Patients who have received neoadjuvant chemotherapy, male patients and recurrent cases were excluded from this study.

Specimens of breast with tumor mass were received by department of Pathology and sections from specimen were taken and tissue processing was done followed by routine haematoxylin and eosin (H&E) staining on histopathology. Scoring was done on these H&E stained slides by Modified Bloom-Richardson grading system. Sections from representative area of tumour mass was subjected to IHC by Bio SB kit for estrogen receptor

(ER) and progesterone receptor (PR ) status and scores were calculated by Allred and H scoring system.. Then, derived scores were compared with the Modified Bloom-Richardson scoring system. Case of IDC (NOS type) breast carcinoma was used as positive external control and normal or non neoplastic epithelium was used as positive internal control. Scores were calculated according to Allred and H scoring system after counting 500 cells. On the basis of proportion and intensity of staining scores were calculated by Allred and H scoring system.

**ALLRED SCORING SYSTEM<sup>9</sup>**

**Proportion score:**

Score	Percentage of stained cells
0	No staining
1	1% nuclei staining
2	1-10% nuclei staining
3	11-33% nuclei staining
4	34-66 %nuclei staining
5	67-100% nuclei staining

**Intensity score:**

Score	Intensity of staining
0	Negative
1	Weak
2	Intermediate
3	Strong

**Allred score (total score) = Proportion score + intensity score**

**0-2 = Negative**

**3-8 = Positive**

**H (HISTOCHEMICAL) SCORING SYSTEM<sup>10</sup>**

SCORE	INTENSIY OF STAINING
0-50	Negative
51-100	Low positive
101-200	Moderate positive
201-300	Strong Positive

**Total score:**  $\Sigma (1 \times [\% \text{ cells with weak staining}] + 2 \times [\% \text{ cells with moderate staining}] + 3 \times [\% \text{ cells with strong staining}]$

**STATISTICAL ANALYSIS**

Analysis of the data was done by IBM SPSS version 20. Kappa statistics was used to observe the agreement between Allred and H scoring system. p value less than 0.05 was considered as statistically significant.

The kappa statistics were interpreted based on the following values : 0-0.2 No agreement, 0.21-0.39 minimal agreement, 0.40-0.59 weak agreement, 0.60-

0.79 moderate agreement, 0.80-0.90 strong agreement, above 0.90 almost perfect agreement.

**RESULTS**

Out of total 60 cases received, 46 (76.6 %) were MRM specimens while 14 (23.4%) were of Trucut biopsy. The results of histopathology and IHC was evaluated and depicted in tabular form with utmost care. The most common age group in the study population was between 48-50 years (38.3%) while the lowest proportion of patients(1.7%) were seen in the >70-year-old age group. Based on the Modified bloom Richardson (BR) grading, most patients belonged to Grade-II (58.3%) followed by Grade-III (28.3%) and least were in Grade-I (13.3%).

**Table 1: Distribution of study population based on age groups**

Age groups (in completed years)	Frequency
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31-40	12(20.0%)
41-50	23(38.3%)
51-60	13(21.7%)
61-70	11(18.3%)
71-80	1(1.7%)
Total	60

**Table 2: Distribution of study population based on the NMBR grading**

Grade	Frequency
1.0	8(13.3%)
2.0	35(58.3%)
3.0	17 (28.3%)
Total	60

Out of 35 cases (58.3%) of Grade-II, 23 (38.3 %) cases are ER+PR+, 7 cases(36.8%) are Triple negative followed by 06 cases (10%) in ER-PR-Her2+. Out of 17 cases (28.3%) of Grade-III ,maximum cases 11 (57.8%) are triple negative followed by ER-PR-Her2+ cases and only 02 cases (3.3%) in ER+PR+ category. Out of 08 cases (13.3%) of Grade-I, 5 cases were ER+PR+, 01 cases (5.2%) belong to Triple negative therefore maximum cases of Grade-I were ER+PR+.

**Table 3 : Distribution of cases according to Grade and ER and PR receptor status**

Grade	ER+PR+	ER+PR-	ER-PR+	ER-PR-Her2+	ER-PR-Her2- (Triple Negative)	Total
1	5 (8.3 %)	0 (0%)	0	2 (3.3%)	1 (5.2%)	8
2	22 (36.6 %)	0 (0%)	0	6(10 %)	7 (36.8%)	35
3	2 (3.3%)	1 (1.6%)	0	3 (5%)	11 (57.8%)	17
Total	30	1(1.6%)	0	10 (16.6 %)	19(31.6%)	60

By Allred scoring for ER receptor, lower BR grades showed higher ER receptors while higher BR grades showed lower ER receptors. Therefore, significant inverse association is found between Allred score and Modified Bloom Richardson Grade. The differences were statistically significant (P=0.042). On Allred scoring for PR receptor , lower BR grades showed higher PR receptors while higher BR grades showed lower PR receptors, although the differences were not statistically significant (P=0.052).

**Table 4 : Association of Allred Score of Estrogen receptor with NMBR grade of tumor mass**

Allred score system	Tumour grading			Chi square test	P value
	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)		
0-1	3 (5)	12 (20)	14 (23.3)	13.090	0.042*
2-3	0	3 (5)	0		
4-6	1 (1.7)	3 (5)	1 (1.7)		
7-8	4 (6.7)	17 (28.3)	2 (3.3)		
Total	8 (13.4)	35 (58.3)	28.3)		

\*Statistically significant

**Table 5: Association of Allred Score of progesterone receptor with NMBR grade of tumor mass**

Allred score system	Tumour grading			Chi square test	P value
	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)		
0-1	3 (5)	13 (21.7)	15 (25)	12.500	0.052*
2-3	1 (1.7)	4 (6.7)	0		
4-6	1 (1.7)	6 (10)	1 (1.7)		
7-8	3 (5)	12 (20)	1 (1.7)		
Total	8 (13.3)	35 (58.3)	17 (28.3)		

On H score for ER, lower NMBR grades showed higher ER receptors while higher NMBR grades showed lower ER receptors. The differences were however not statistically significant (p=0.119\*). On H score for PR, lower NMB grades showed higher PR receptors while higher NMBR grades showed lower PR receptors. The differences were however not statistically significant (p=0.135\*)

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**Table 6: Association of H score of estrogen receptor with NMBR grading of tumour mass**

Division of H score system	Tumour grading			Chi square test	p value
	Grade1(%)	Grade 2(%)	Grade 3(%)		
0-50	4 (6.7)	16 (26.7)	15 (25)	10.142	0.119*
51-100	0	1 (1.7)	0		
101-200	1 (1.7)	9 (15)	1 (1.7)		
201-300	3 (5)	9 (15)	1 (1.7)		
Total	8 (13.4)	35 (58.3)	17 (28.3)		

**Table7 :Association of H score of Progesterone receptor with NMBR grading of tumor mass**

Division of H score system	Tumour grading			Chi square test	p value
	Grade I N (%)	Grade II N (%)	Grade III N (%)		
0-50	4	16	15	9.764	0.135*
51-100	0	1	0		
101-200	1	8	1		
201-300	3	10	1		
Total	8 (13.4)	35 (58.3)	17 (28.3)		

**Table 8 :Agreement between detection of estrogen receptors by Allred and H scores**

H score	Allred score	
	Positive N (%)	Negative N (%)
Positive	25	0
Negative	5	30
Total	30	30

Agreement between	Kappa	P value
ER detection by Allred and HR scores	.833	.000*

Kappa statistics shows an almost perfect agreement (k=0.833) between the Allred and H score in terms of detection of ER. The level of agreement was found to be statistically significant.

**Table 9: Agreement between detection of progesterone receptors by Allred and H scores**

H score	Allred score	
	Positive	Negative

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	N (%)	N (%)
Positive	20	0
Negative	9	31
Total	29	31

Agreement between	Kappa	P value
PR detection by Allred and HR scores	.263	.000*

Kappa statistics show a fair agreement (k=0.263) between the Allred and H score in terms of detection of PR. The level of agreement was found to be statistically significant.

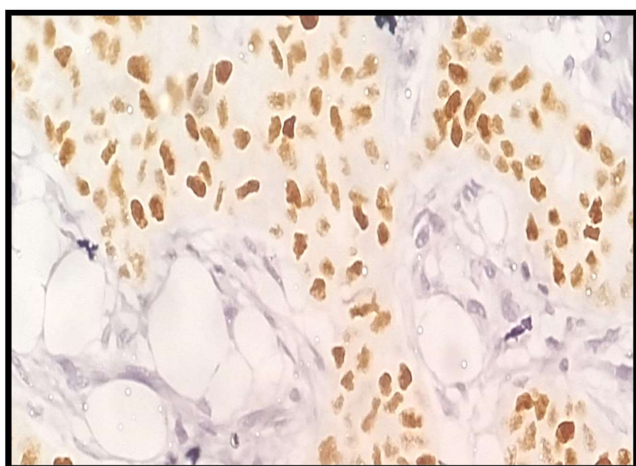


Figure 1: Section stained with immunohistochemistry stain (ER) shows 34-66% nuclei suggesting proportion score 4 with intermediate staining intensity suggesting intensity score 2 at high Power(x40) Allred score:6 and H score =199 (moderate positive) (X400)

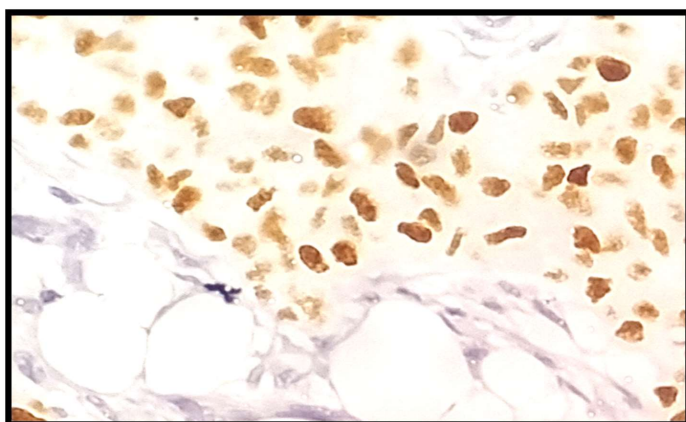


Figure2 : Section stained with immunohistochemistry stain (PR) shows34-66% nuclei stained positive suggesting proportion score 4 with intermediate staining intensity suggesting intensity score 2 with Allred score:6 (X400)

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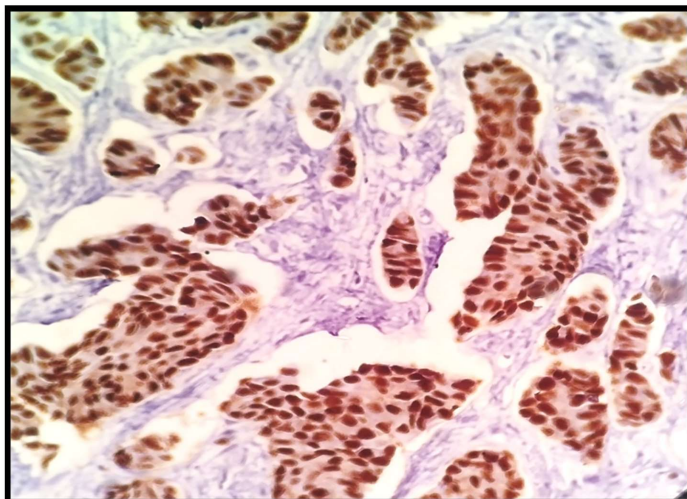


Figure 3 : Section stained with immunohistochemistry stain (ER) 70-100% nuclei suggesting proportion score 5 with strong staining intensity suggesting intensity score 3 with Allred score:8 and H score:224 (X400)

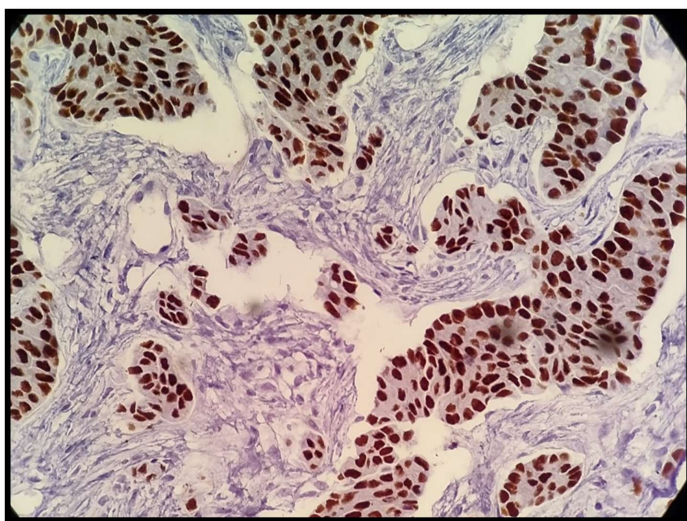


Figure 4: Immunohistochemistry for PR-80-90% cells show strong positivity with Allred score 8 and H score :254 (X400)

#### DISCUSSION

Breast malignancy is a hormone dependent tumor. Endocrine therapy is very helpful in those tumors which do express the estrogen and progesterone receptor. Hormone receptor analysis is prerequisite now a days before starting adjuvant therapy since it is easier to perform and it also has higher sensitivity. Many methods have been used to evaluate ER expression in invasive breast tumours for almost 40

years. The IHC is the method that is most frequently employed nowadays. It is simple, cheap, and sensitive, and it may be done on tissues that have been paraffin-processed and fixed in formalin. Since ER controls PR expression, the presence of PR indicates that the ER route is active. PR becomes active due to progesterone, which encourages the growth of cancer cells.<sup>11,12</sup>

The mean age at presentation was 50.4 years. The youngest patient's age that presented with breast

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carcinoma was 32 years, while the oldest was 71 years. Patnayak et al<sup>13</sup> reported that the age range was 23-90 years with a mean of 50.7 years. Another retrospective study by Ambrose et al,<sup>14</sup> in 321 female invasive breast carcinomas, reported ages between 24 to 99 years, with a mean of 53.8 years. Desai et al,<sup>15</sup> in their study on estrogen and progesterone receptor (ER & PR) status of breast cancer in the Indian population, found that most patients (63.4%) in their cohort of breast carcinoma, to be pre-menopausal (<50 years old). Mean age of Indian breast cancer patients is found to be lower when compared to the Western countries with an average difference of one decade.<sup>16,17</sup>

In Present study, based on the Modified bloom Richardson grading, most patients belonged to Grade-II (58.3%) followed by Grade-III (28.3%) and least were in Grade-I (13.3%). Similar finding was found in study

of Rai PD et al<sup>10</sup> with most cases of Grade II (73.8% ), Pingle Prathyusha et al<sup>18</sup> with 36% cases of Grade II , Ann George et al<sup>19</sup> with majority of cases belong to Grade-II (46%) followed by Grade-III (34%) and least of Grade-I (20%) and Pathak TB et al<sup>20</sup> with 59% cases of Grade II and least of Grade-I (20%). In contrast most patients in the study by Desai et al,<sup>21</sup> were high-grade (Grade III) tumours. In their study from South India, Ambrose et al,<sup>22</sup> found two-thirds of the patients with grade-1 or 2, and one-third in Grade-III. Breast cancer patients of Indian origin tend to be younger; tumours are often large when first diagnosed, and of a high grade as compared to Western series.<sup>21</sup>

In present study maximum cases (95%) cases were reported as invasive ductal carcinoma, NOS and 1.6 % cases each of Mucinous carcinoma, invasive micropapillary Carcinoma and Medullary carcinoma.

Almost similar finding was found in following studies:

	<b>Invasive ductal Carcinoma ,NOS</b>	<b>Mucinous Carcinoma</b>	<b>Medullary carcinoma</b>	<b>Invasive Micropapillary carcinoma</b>
<b>Pingle Prathyusha et al<sup>18</sup></b>	92%	1.3%	1.3%	1.3%
<b>Mittal A et al<sup>23</sup></b>	83.8%	9.6%	1.6%	1.6%
<b>Chinnam A et al<sup>24</sup></b>	82.6%	0.2% (2 cases)	0.2% (2 cases)	2.9% (6 cases)
<b>Pathak TB et al<sup>20</sup></b>	96%	0.7 %	1.4%	0.7%
<b>Present study</b>	95%	1.6%	1.6%	1.6%

In the present study more than half of the patients (52.2%) presented with axillary nodal metastasis at the time of the biopsy, while 47.8% had no nodal involvement. Similar finding was observed by Patnayak et al<sup>1</sup> who conducted study on 309 patients of breast carcinoma and examined lymph nodes of 211 patients and noted metastatic lymph nodes in 65.4% cases. In developed countries, majority of the patients have a negative lymph node status at presentation compared to Indians, which can be attributed to the early detection programs prevalent in the western countries.<sup>25,26,27,28</sup>

There were 05 cases with Allred score positive and H score Negative for ER Receptors along with 09 cases for PR receptors for which Allred score was Positive and H score was negative.

It was mainly observed in 1–10% of positively stained cells. This finding was approximately comparable with other studies, i.e., Brouckaert et al.<sup>28</sup> Qureshi and Pervez,<sup>29</sup> and Vijayashree et al.<sup>30</sup> This discordance is mainly observed when 1-10% of cells stain positive. This can be explained by study of Rai PD et al<sup>10</sup> due to assessment of staining intensity visually which is susceptible to subjective variation and may lead to interobserver differences in the interpretation of results. It may also occur from using different antibodies with variable sensitivity, particularly when hormone receptor is low.

Kappa statistics shows almost perfect agreement (k=0.833) between the Allred and H score in terms of detection of ER. The level of agreement was statistically

significant. Kappa statistics show fair agreement (k=0.263) between the Allred and H score in terms of detection of PR. The level of agreement was however, not found to be statistically significant.

**LIMITATIONS**

The Allred score combines the percentage of positive cells and the intensity of the staining, which can lead to variability in interpretation between different pathologists. The study was carried out in a single institution and therefore the generalisability of the findings is limited. The small sample size could have resulted in low statistical power of the study.

**CONCLUSION**

In our study, quantification of ER/PR expression by both H score and Allred score methods produced comparable results. When compared against each other, both the ER and PR show a high level of agreement between them. There was statistically significant positive correlation of both scoring system with NMBR Grading system. Low hormone receptor positivity is associated with advanced stage at presentation, and higher grade in our population. Both being semiquantitative methods and can be practiced regularly. However Allred scoring system is quick and widely accepted for breast carcinoma.

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