OPEN ACCESS
Saudi Journal of Pathology and Microbiology
Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) |ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Study of HER2/neu over Expression and Androgen Receptor Status in Prostate Neoplasm - Correlating with Histological Grading

Liza Das[1*], Ramesh Chandra Mohanty[1], Raghumani Mohanty[2], Sarata Chandra Singh[3]

[1]Department of Pathology, Hi-Tech Medical College, Utkal University, Bhubaneswar, Odisha, India
[2]Department of Pathology, Hi Tech Medical College, Utkal University, Cuttack, Odisha, India
[3]Department of General Medicine, SCB Medical College, Utkal University, Cuttack, Odisha, India

DOI: 10.36348/sjpm.2021.v06i09.008 | **Received:** 11.08.2021 | **Accepted:** 17.09.2021 | **Published:** 21.09.2021

***Corresponding author:** Dr. Liza Das

Abstract

Prostate cancer is the second most common cancer and sixth leading cause of cancer death among men worldwide, with an estimated recorded number of 1.1 million cases and 307,000 deaths which allocates 15% of all new cases of cancer in men. Androgen receptor (AR) expression is maintained throughout prostate cancer progression, and the majority of androgen –independent or hormone refractory prostate cancers express Androgen receptor. HER2/neu, a biological marker named human epidermal growth factor receptor -2[EGFR-2] is also known as c-erb-2 plays a major role in understanding the oncogenesis of prostate adenocarcinoma and has a significant prognosis in the disease. ***Aim of The Study*:** To study the histomorphological features and grading of prostatic neoplasms with Gleason's scoring. To study over the expression of HER2/neu & androgen receptor status in prostate neoplasm. To study the correlation of Gleason's scoring with AR & HER2/neu expression in prostate cancer. All patients who were diagnosed to have prostatic cancer and PIN and were diagnosed by prostatic biopsy either by prostatectomy, TURP or Trucut procedure were included in the study and were examined for analyzing various histomorphological features and grading of various neoplasms of prostate with correlation to overexpression of immmunohistochemical markers Her2/neu and AR. ***Conclusion*:** This study may help the clinician in finding out her2/neu over expressed cases and implementing anti her2/neu therapy along with androgen deprived therapy for longer survival of prostate cancer patients although further studies on larger series of prostate carcinoma is required for new generation of more efficient anti her2 drugs.

Keywords: Prostate cancer, HER2/neu, Androgen receptor, Histopathology, Gleason's Score.

INTRODUCTION

Prostate cancer is the second most common cancer and sixth leading cause of cancer death among men worldwide, with an estimated recorded number of 1.1 million cases and 307,000 deaths which allocates 15% of all new cases of cancer in men [1]. The normal development and maintenance of prostate is dependent on androgen acting through the androgen receptor [AR]. AR expression is maintained throughout prostate cancer progression, and the majority of androgen –independent or hormone refractory prostate cancers express AR. AR expression is maintained throughout prostate cancer progression, and the majority of androgen –independent or hormone refractory prostate cancers express AR [2]. HER2/neu, a biological marker named human epidermal growth factor receptor -2[EGFR-2] is also known as c-erb-2 plays a major role in understanding the oncogenesis of prostate adenocarcinoma and has a significant prognosis in the disease. HER2/neu over expression induces transactivation of androgen receptor pathway, leading to advance stage of prostate cancer [3]. Hormonal therapy has been the mainstay of treatment for advanced prostate cancer for over five decades but treating alone with anti-androgen therapy is not successful in these cases so targeting for anti HER2/neu can be used as targeted therapy is required for better survival and prognosis [4].

The present study was done to evaluate the Her2/neu over expression and Androgen Receptor status in prostate neoplasms through immunohistochemistry and correlating it with the histomorphology along with its grading, in the specimen from transurethral resection of prostate, Trucut biopsy or prostatectomy with the aim of providing the clinician a conclusion for a better therapeutic hormonal management strategy.

Citation: Liza Das *et al* (2021). Study of HER2/neu over Expression and Androgen Receptor Status in Prostate Neoplasm - Correlating with Histological Grading. *Saudi J Pathol Microbiol, 6*(9): 316-322.

AIM OF THE STUDY

1. To study the histomorphological features and grading of prostatic neoplasms with Gleason's scoring.
2. To study over the expression of HER2/neu & androgen receptor status in prostate neoplasm.
3. To study the correlation of Gleason's scoring with AR & HER2/neu expression in prostate cancer.

MATERIAL AND METHODS

This was a two years prospective study conducted in the Department of Pathology and Urology from November 2016 to October 2018 in Hi-Tech Medical College, Bhubaneswar, Odisha, India. All patients who were diagnosed to have prostatic cancer and PIN and were diagnosed by prostatic biopsy either by prostatectomy, TURP or Trucut procedure were included in the study. Inflammatory lesions of the prostate and benign hyperplasia of prostate were excluded from the study. After receiving the sample, it was examined macroscopically as regards the size, shape, haemorrhage, necrosis or any specific gross feature. The tissue bits or sectioned tissue were processed in automated tissue processor. 5µ thick Sections were stained with Haematoxylin and Eosin stain and 3µ thick sections were stain with immunostaining. Histological diagnosis was made and gleason's grading score was given followed by immunological staining. Immunostaining was done by using biogenex markers. Positive control for Her2/neu was taken from cases of breast carcinoma. Positive control for AR was taken from cases of BHP normal prostate. Negative external control was used by omitting the primary antibody and treated with phosphate-buffered saline (PBS). Her2/neu can be both membranous and cytoplasmic in location, in prostate cancer they are mostly cytoplasmic in location. AR is nuclear in localization. Microscopy of Prostatic neoplasms: 1) Histological type: a) Adenocarcinoma(conventional), b) Others (specify) ;2) Histological grade: Gleason pattern- Primary (predominant) pattern-Grade1;,Grade2; Grade3; Grade4; Grade 5. Secondary (worst Remaining) Pattern-Grade1; Grade2; Grade3; Grade4; Grade 5-Total Gleason score 3) PIN: present /absent - If present low grade /high grade 4) perineural invasion :present/absent 5)Lymphatic invasion: present /absent 6) Other associated findings: BPH/AAH/etc.

Interpretation of immmunohistochemical staining for Her2/neu [5]

Staining pattern	**Score**	**Her2/Neu protein over expression Assessment**
No staining or membrane staining observed in < 10% of tumor cells.	0	Negative
Faint or barely perceptible membrane staining detected in > 10% of tumor cells: cells are stained only in part of their membrane	1	Negative
Weak to moderate, complete membrane staining observed in > 10% of tumor cells	2	Weakly positive
A Strong complete membrane staining observed in > 30 %(formerly10%) of tumor cells.	3	Strongly positive

Her2/Neu protein over expression was interpreted as positive /negative. Positive staining was defined as positive staining of membranous or cytoplasmic of epithelial cells, overexpression said when the staining intensity is 2+ or 3+ **[**6].

AR stain interpretation [7]:

SCORE FOR AR	**INTENSITY OF IMMUNOSTAINING**
0	No staining
1+	Weak equivocal staining
2+	Moderate unequivocal staining
3+	Strong staining

AR is a nuclear stain that taken up by both stromal and epithelial cells in BHP and prostate carcinoma, so positive in both only pattern and intensity is different. Carcinoma exhibit heterogeneous staining and BHP shows homogenous staining. AR stain interpretation is negative when no staining is seen.

The clinical, histological and immunohistochemical data were collected. Then transformed to a master chart by using Microsoft excel sheet ,which was then subjected to statistical analysis using Chi-square test by using SPSS,version 20. The findings were arranged in tables and graphs using Microsoft excel sheet. Analysis was done in form of percentage and proportion and represented as tables and figures where necessary p value of <0.05 is considered as statistically significant.

RESULTS

This was a two years prospective study conducted in the Department of Pathology and Urology from November 2016 to October 2018 in Hi-Tech Medical College, Bhubaneswar, Odisha, India. In the present study there were 45 cases identified as neoplastic lesions of prostate in the age group of 30-90yrs (Table-1). Most of the patients presented with increase in frequency in 11 cases (24.44%) and dysuria in 9 cases (20%) due to obstructive urinary tract symptoms (Table-2).

Table 1: Distribution of cases according to the age group

SL.NO	AGE GROUP	FREQUENCY	PERCENTAGE
1	<30yrs	_	_
2	30-39yrs	1	2.22%
3	40-49yrs	_	_
4	50-59yrs	6	13.33%
5	60-69yrs	15	33.33%
6	70-79yrs	17	37.77%
7	80-89yrs	6	13.33%
8	>90yrs	_	_
	TOTAL	45	100%

Table 2: Clinical presentation of prostatic neoplastic lesion

Clinical symptoms	Present Study(N=45)
Frequency	11(24.44%)
Nocturia	3(6.66%)
Incomplete voiding	5(11.11%)
Difficulty in voiding	6(13.34%)
Urinary retention	4(8.88%)
Hematuria	5(11.11%)
Dysuria	9(20%)
Bone pain	2(4.45%)
Total	45

Needle core biopsy procedure (Trucut) comprised the majority of samples received i.e. 23(51.11%) cases followed by prostatic chips (TURP) in 20(44.44%) cases and enbloc removal of prostate in 2(4.45%) of cases (Fig-1). Total no 0f 45 cases were studied, out of which 5 cases belonged to Prostatic Intraepithelial Neoplasm (PIN) and 40 cases were prostatic adenocarcinoma. PIN was associated in 3 cases of BHP. All the adenocarcinoma cases were detected to be acinar type (Fig-2).

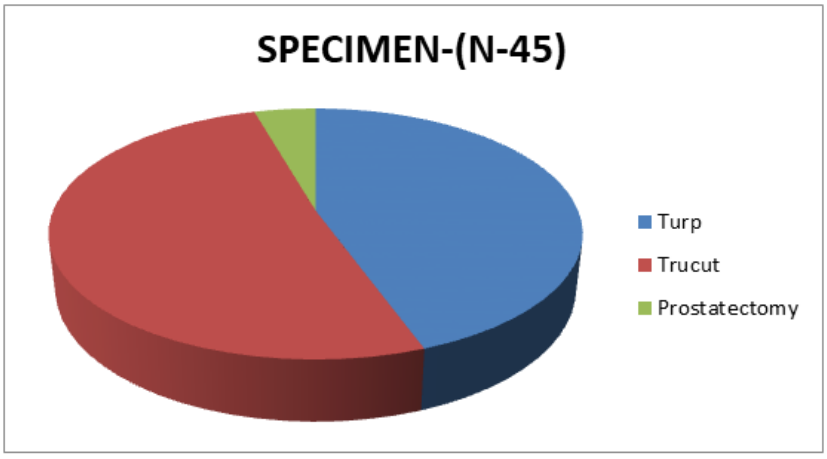

Fig 1: Pie chart showing types of specimen obtained in prostatic neoplasms

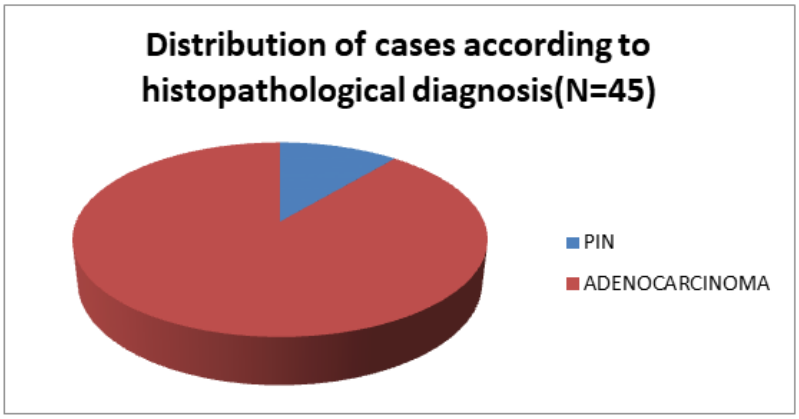

Fig 2: Distribution of cases of prostatic neoplasms according to Histopathological diagnosis

The Gleason score≤6 (Grade-1) constituted 9(22.5%) cases, 6(15%) cases was in gleason score7(3+4=Grade2),1(2.5%) case in Gleason score 7(4+3=grade 3), Gleason score8(4+4=grade 4) seen in 7(17.5%) of cases and most of the cases diagnosed in grade 5(Gleason score 9 and 10) constituted 42.5% of cases(17) followed by grade-1 22.5% of (9) Cases (Table-3).

Table 3: Distribution of Prostatic Adenocarcinoma according to Gleason Grading system (N=40)

HISTOLOGICAL GRADE	NO 0F CASES	PERCENTAGE(%)
GRADE-1(GS≤ 6)	9	22.5%
GRADE-2(GS-3+4)	6	15%
GRADE-3(GS-4+3)	1	2.5%
GRADE-4 (GS-8)	7	17.5%
GRADE-5(GS-9,10)	17	42.5%
Total	40	100%

Immunochemistry was done using Her2/neu in 5(11.11%) cases of Prostatic intraepithelial neoplasia, and 40(88.89%)cases of adenocarcinoma. Present study showed Her2/neu negativity in all 5(100%) cases of PIN and out of 40 cases of adenocarcinoma 5(12.5%) cases show over expression of Her2/neu. Out of 5 cases of Her2/neu positivity 1(2.5%) case showed moderate expression and 4(10%) cases showed strong expression of Her2/neu (Table-4).

Table 4: Expression of Her2/neu immuno staining in different cases of prostatic neoplasms

Her2/neu Expression	**PIN(5)**	**Adenocarcinoma Cases(40)**	**Over all Cases(45)**	
Negative	5(100%)	35(87.5%)	40(88.89%)	
Positive:				
Weak(1+)	_	_	_	
Moderate(2+)	_	1(2.5%)	1+(2.22%)	
Strong(3+)	_	4(10%)	4+ (8.89%)	
Total Positive		5(12.5%)	5(11.11%)	

In the present study out of 45 cases only 5 (11.11%)cases showed over expression of Her2/neu including 1 case in grade-4 and 4 cases in grade-5(9,10)prostate adenocarcinoma.Rest of the 40 cases were negative (Table-5).

Table 5: Her2/neu positivity correlating with histological grading

PIN AND HISTOLOGICAL GRADING OF TUMOURS	NO OF CASES	HER2/NEU EXPRESSION		
		1+ (<10%)	2+ (>10%)	3+ (>30%)
PIN	5	_	_	_
GRADE-1	9	_	_	_
GRADE-2	6	_	_	_
GRADE-3	1	_	_	_
GRADE-4	7	_	_	1(2.22%)
GRADE-5	17	_	1(2.22%)	3(6.67%)
TOTAL	45	POSITIVE =5(11.11%)CASES		

P value=0.375 Chisquare=5.35

In present study 0ut 0f 45 cases 44(97.78%) cases showed AR expression of variable intensity, 1(2.22%) case was negative for AR. All 5 (100%) PIN cases showed AR positivity and 39(97.5%) cases of adenocarcinoma showed AR expression out of which 5(12.5%) cases showed weak positivity, 13(32.5%) cases showed moderate positivity, 21(52.5%) cases showed strong positivity and 1(2.5%) case is negative for AR. Stromal cells surrounding or near about the malignancy epithelial cells showed negative AR staining Where as stromal cells in cases of PIN showed uniform stromal positivity (Table-6).

Table 6: Expression of AR immunostaining in different cases of prostatic neoplasms

AR Expression	PIN(5)	Adenocarcinoma Cases(40)	Over all Cases(45)
Negative	_	**1(2.5%)**	**1(2.22%)**
Positive:			
Weak(1+)	_	**5(12.5%)**	**5(11.11%)**
Moderate(2+)	**3(60%)**	**13(32.5%)**	**16(35.56%)**
Strong(3+)	**2(40%)**	**21(52.5%)**	**23(51.11.%)**
Positive	**5(100%**	**39(97.5%)**	**44(97.78%)**

P value=0.608 Chisquare=1.83

In present study AR expression is maintained throughout the tumour progression and 5 PIN cases showed 3 moderate and 2 strong positivity, 9 (grade 1) cases showed 3 moderate and 6 strong positivity, 6 (grade 2)cases showed 4 moderate and 2 strong positivity, 1(grade- 3) case showed moderate positivity, 7 (grade-4) cases showed 1 weak , 1moderate and 4 strong positivity and 17 (grade-5) cases showed 1 negative,4 weak ,4 moderate and 8 strong positivity cases**.** In prostate carcinoma malignant epithelial cells showed heterogeneous positivity i.e some cells showed negativity where as other cells showed high expression of AR demonstrated by intensity, irrespective of Grade out of 40 cases of adenocarcinoma of prostate 34 (85%)cases showed either 2+ or 3+ AR immunoexpression. In the present study there were 24(60%) cases belonging to Grade-4 and Grade -5 out of which 18(45%) cases showed AR positivity of 2+(5 cases) and 3+(13 cases) (Table-7).

Table 7: Distribution, frequency and intensity of AR according to histological grading

PIN AND HISTOLOGICAL GRADING OF TUMORS	NO OF CASES	AR EXPRESSION			
		0	1+	2+	3+
PIN	5	_	_	3	2
GRADE-1	9	_	_	3	6
GRADE-2	6	_	_	4	2
GRADE-3	1	_	_	1	
GRADE-4	7	_	1	1	5
GRADE-5	17	1	4	4	8
TOTAL	45	1(2.2%)	5(11.1%)	16(35.6%)	23(51.1%)

P value =0.55 Chisqure=13.69

In the present study Grade 1,2,3 constituted 16 cases where Her2/neu were negative and AR showed variable positivity . Similarly in Grade -4 there are 7 cases out of which 1 case showed 3+ her2 positivity which were also positive for AR.In Grade -5 there are 17 cases of which 4 were Her2/neu positivity (2+,3+) and all except 1 showed AR positivity of variable intensity (Table-8).

Table 8: Correlation of Her2/neu over expressed cases and AR to histological grade

GRADE	NO OF CASES	GLEASON SCORE	HER2/NEU POSITIVE CASES ACCORDING TO INTENSITY	INTENSITY SCORE OF AR EXPRESSION
GRADE-1,2,3,	16	<8	Her2/neu Negativity	AR positivity(2+,3+)
GRADE-4	7	8	1(3+)	Weak (1+)
GRADE-5	17	9	1(3+)	Strong(3+)
			1(3+)	Strong(3+)
		10	1(2+)	Weak(1+)
			1(3+)	Strong(3+)

DISCUSSION

The present study was carried out on 45 cases of TURP, Trucut and prostatectomy specimens and the specimen were examined for analyzing various histomorphological features and grading of various neoplasms of prostate with correlation to overexpression of immmunohistochemical markers Her2/neu and AR. The cases include were PIN and adenocarcinoma of prostate.

Her 2/neu overexpression showed a wide range of variation 1.51% to 34% in the different studies compared. Present study showed overexpression in 5(11.11%) cases of prostate carcinoma and one case showed moderate positivity and 4 cases showed strong positivity, which is similar to the findings of Koeppen *et al*., [8] and Lara *et al*., [9] where they found 8.2% and 8% respectively. Kwang Hyun Baek *et al.*, [10] showed lowest expression of her2/neu 1.51% in above studies and highest expression 34% seen in Kuhn *et al*., [11].

Present study showed her2/neu over expression in 11.11% cases and the cases belonged to Grade-4 and 5 only and no expression seen in Grade 1 to 3. Her 2 neu over expression seen in higher grade and correlating higher Gleason grade. These results confirm the findings of Sarah minner *et al*., [12], Signoretti *et al*., [13] and Shi Y *et al*., [14] Other authors Kwang *et al*., [15], Primo Lara *et al*., [16], Joan Charles *et al*., [17] showed similar expression but no correlation with the histological grading.

Out of 45 cases of prostatic neoplasms which include 5 cases of PIN and 40 cases of adenocarcinoma, AR expression was observed in 44 cases (97.77%). It was comparable with other studies in the above table. In the present study, AR expression was observed in 100% and 97.5% in PIN and adenocarcinoma cases respectively. The frequency of AR expression is heterogeneous in adenocarcinoma in comparison to BHP control case which showed homogenous expression in both epithelial cells and stroma cells. Adenocarcinoma cases show weak expression of AR in the stroma surrounding the malignant glands. AR expression in adenocarcinoma was of variable intensity in the highest Gleason score so this association are not statistically significant. Similar findings were documented in the studies with Kwang *et al*., [15] and Qui *et al*., [18] where as other authors Wesam M Osman *et al*., [19] and Husain I *et al*., [7] in their studies had shown inverse correlation between AR expression and Gleason score.

It was observed in the present study that there were 17 cases of high grade prostatic carcinoma (grade 5) of which 4 cases showed Her 2/neu positivity (3+, 2+) and with variable AR expression. This identifies a subset of cases of prostatic carcinoma where high expression of both Her2/neu and AR in combination has an adverse prognosis as reported by Ricciardelli C *et al*., [20]. This group of patients with high AR expression need Anti androgenic therapy as reported by Pertschuk *et al*., [21].

CONCLUSION

AR heterogeneous positivity is seen in prostatic carcinoma along with absence of peritumoral stromal positivity. Her2/neu over expression in high grade tumors may indicate that her2/neu oncoprotein is involved in stimulation of the growth and oncogenic transformation of prostatic cells and progression of prostatic carcinoma. Her2/neu over expression in prostate cancer may result from transcriptional and posttranscriptional mechanism. Over expression of Her2/neu receptor tyrosine kinase is associated with high grade prostate carcinoma and progression to androgen independence. High grade prostate carcinoma shows both AR and Her2/neu positivity. Increased Her2/neu expression may potentially lead to an aggressive behavior of tumor cells. Thus therapeutic targeting of this tyrosine kinase receptor in prostate cancer may be warranted /suggested.

This study may help the clinician in finding out her2/neu over expressed cases and implementing anti her2/neu therapy along with androgen deprived therapy for longer survival of prostate cancer patients although further studies on larger series of prostate carcinoma is required for new generation of more efficient anti her2 drugs.

REFERENCES

1. Sadeghi-Gandomani, H., Yousefi, M., Rahimi, S., Yousefi, S., Karimi-Rozveh, A., Hosseini, S., ... & Salehiniya, H. (2017). The incidence, risk factors, and knowledge about the prostate cancer through worldwide and Iran. *World Cancer Research Journal*, *4*(4), 1-8.
2. Heinlein, C. A., & Chang, C. (2004). Androgen receptor in prostate cancer. *Endocrine reviews*, *25*(2), 276-308.
3. Mohler, J. L. (2008). A role for the androgen-receptor in clinically localized and advanced prostate cancer. *Best practice & research Clinical endocrinology & metabolism*, *22*(2), 357-372.
4. Carles, J., Lloreta, J., Salido, M., Font, A., Suarez, M., Baena, V., ... & Fabregat, X. (2004). Her-2/neu expression in prostate cancer: a dynamic process?. *Clinical cancer research*, *10*(14), 4742-4745.
5. Rosai &Ackerman. (2004). Surgical pathology, 10th edition. Missouri: Mosby.
6. Jenster, G. (1999, August). The role of the androgen receptor in the development and progression of prostate cancer. In *Seminars in oncology*, 26(4), 407-421.
7. Husain, I., Shukla, S., Soni, P., & Husain, N. (2016). Role of androgen receptor in prostatic neoplasia versus hyperplasia. *Journal of cancer research and therapeutics*, *12*(1), 112-116.
8. Koeppen, H. K. W., Wright, B. D., Burt, A. D., Quirke, P., McNicol, A. M., Dybdal, N. O., ... & Hillan, K. J. (2001). Overexpression of HER2/neu in solid tumours: an immunohistochemical survey. *Histopathology*, *38*(2), 96-104.
9. Lara Jr, P. N., Meyers, F. J., Gray, C. R., Gandour Edwards, R., Gumerlock, P. H., Kauderer, C., ... & Gandara, D. R. (2002). HER- 2/neu is overexpressed infrequently in patients with prostate carcinoma: Results from the California Cancer Consortium screening trial. *Cancer*, *94*(10), 2584-2589.
10. Baek, K. H., Hong, M. E., Jung, Y. Y., Lee, C. H., Lee, T. J., Park, E. S., ... & Lee, S. W. (2012). Correlation of AR, EGFR, and HER2 expression levels in prostate cancer: immunohistochemical analysis and chromogenic in situ hybridization. *Cancer research and treatment: official journal of Korean Cancer Association*, *44*(1), 50-56.
11. Kuhn, E. J., Kurnot, R. A., Sesterhenn, I. A., Chang, E. H., & Moul, J. W. (1993). Expression of the c-erbB-2 (HER-2/neu) oncoprotein in human prostatic carcinoma. *The Journal of urology*, *150*(5 Part 1), 1427-1433.
12. Minner, S., Jessen, B., Stiedenroth, L., Burandt, E., Köllermann, J., Mirlacher, M., ... & Schlomm, T. (2010). Low level HER2 overexpression is associated with rapid tumor cell proliferation and poor prognosis in prostate cancer. *Clinical Cancer Research*, *16*(5), 1553-1560.
13. Signoretti, S., Montironi, R., Manola, J., Altimari, A., Tam, C., Bubley, G., ... & Loda, M. (2000). Her-2-neu expression and progression toward androgen independence in human prostate cancer. *Journal of the National Cancer Institute*, *92*(23), 1918-1925.
14. Shi, Y. A. N., Brands, F. H., Chatterjee, S., Feng, A. C., Groshen, S., Schewe, J., ... & Cote, R. J. (2001). Her-2/neu expression in prostate cancer: high level of expression associated with exposure to hormone therapy and androgen independent disease. *The Journal of urology*, *166*(4), 1514-1519.
15. Baek, K. H., Hong, M. E., Jung, Y. Y., Lee, C. H., Lee, T. J., Park, E. S., ... & Lee, S. W. (2012). Correlation of AR, EGFR, and HER2 expression levels in prostate cancer: immunohistochemical analysis and chromogenic in situ hybridization. *Cancer research and treatment: official journal of Korean Cancer Association*, *44*(1), 50-56.
16. Lara Jr, P. N., Meyers, F. J., Gray, C. R., Gandour Edwards, R., Gumerlock, P. H., Kauderer, C., ... & Gandara, D. R. (2002). HER- 2/neu is overexpressed infrequently in patients with prostate carcinoma: Results from the California Cancer Consortium screening trial. *Cancer*, *94*(10), 2584-2589.
17. Carles, J., Lloreta, J., Salido, M., Font, A., Suarez, M., Baena, V., ... & Fabregat, X. (2004). Her-2/neu expression in prostate cancer: a dynamic process?. *Clinical cancer research*, *10*(14), 4742-4745.
18. Qiu, Y. Q., Leuschner, I., & Braun, P. M. (2008). Androgen receptor expression in clinically localized prostate cancer: immunohistochemistry study and literature review. *Asian journal of andrology*, *10*(6), 855-863.
19. Osman, W. M., Abd El Atti, R. M., & Abou Gabal, H. H. (2013). DJ-1 and androgen receptor immunohistochemical expression in prostatic carcinoma: a possible role in carcinogenesis. *Journal of the Egyptian National Cancer Institute*, *25*(4), 223-230.
20. Ricciardelli, C., Jackson, M. W., Choong, C. S., Stahl, J., Marshall, V. R., Horsfall, D. J., & Tilley, W. D. (2008). Elevated levels of HER- 2/neu and androgen receptor in clinically localized prostate cancer identifies metastatic potential. *The Prostate*, *68*(8), 830-838.
21. Pertschuk, L. P., Macchia, R. J., Feldman, J. G., Brady, K. A., Levine, M., Kim, D. S., ... & Greene, G. L. (1994). Immunocytochemical assay for androgen receptors in prostate cancer: a prospective study of 63 cases with long-term follow-up. *Annals of surgical oncology*, *1*(6), 495-503.