

## Relationship between HER-2 Gene Expression and Prognostic Prostate Cancer Parameters in Trans Rectal Ultrasound-guided Biopsies

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ARTICLE INFO	ABSTRACT
<p><i>Article type:</i> Original article</p> <hr/> <p><i>Article History:</i> Received: 16-Feb-2019 Accepted: 05-May-2019</p> <hr/> <p><i>Key words:</i> Gleason score HER2-neu peptide Imaging guided biopsies Prostate cancer</p>	<p><b>Introduction:</b> Prostate carcinoma is the most common cancer among men. Overexpression of HER2-neu gene was reported to affect the growth and prognosis of some tumors. HER2 gene amplification is seen in about one-third of prostatic adenocarcinoma cases. It also seems to associate with androgen independence of the prostate tumors. We evaluated the HER2-neu gene expression in prostate cancer and its relationship with known prognostic factors.</p> <p><b>Materials and Methods:</b> We included 60 patients with prostate carcinoma and assessed them by transrectal ultrasound-guided (TRUS) biopsy. HER2-neu expression was evaluated through pathological examination of TRUS biopsy specimens using immunohistochemical staining. Prognostic factors of prostatic carcinoma including serum PSA, number and percentage of involved cores, Gleason score, Gleason grade, extra-prostatic extension of the tumoral cells, and tumor volume was measured. Data were analyzed in SPSS software and P&lt;0.05 was considered significant.</p> <p><b>Results:</b> Among 60 patients, HER2-neu was negative in 49 (81.7%) cases (35 cases with 0 score and 14 cases with 1+ score) and weakly positive expression (2+ score) was seen in 11 (18.3%). Among evaluated factors, tumor volume was the only one that significantly associated with HER2-neu expression.</p> <p><b>Conclusion:</b> The rate of HER2-neu expression was not high in our patients. Among various variables evaluated in our study, only tumor volume had significant association with the expression of HER2-neu. Therefore, it can be used as a prognostic factor in these patients.</p>
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### Introduction

Prostate cancer (PCa) is the most common malignancy in men and the second lethal cancer, worldwide. It is also the second most prevalent cancer among Iranian men. The chance of having invasive prostate cancer during a man's lifetime is 1 in 6 or 15.6% (1-4).

Factors that underlie tumor pathogenesis and resistance to conventional therapies have been

increasingly studied recently. The human epidermal growth factor receptor 2 (HER2/neu) is an oncoprotein, which belongs to the epidermal growth factor receptor (EGFR) family and plays a major role in cell growth and differentiation (5-7).

The HER2/neu proto-oncogene, located on the 17<sup>th</sup> chromosome, encodes a transmembrane

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tyrosine kinase growth factor receptor whose overexpression was proved to be involved in the development of various malignancies, including non-small-cell lung cancer, colon cancer and breast cancer, in which it may have a prognostic value as well (5). However, the significance of HER2 overexpression in PCa remains controversial. In vitro studies have reported a very wide range for the rates of HER2 gene expression (8, 9).

Several studies reported HER2/neu overexpression to be associated with lower survival rates in PCa patients (10, 11). However, it is unknown whether the alterations in HER2 expression can be considered as a marker of tumor progression and metastasis.

The purpose of this study was to evaluate HER2-neu expression in prostate carcinoma and its association with prognostic factors in transrectal ultrasound (TRUS)-guided prostate core needle biopsies.

## Methods

### *Patient selection and TRUS-biopsy*

This cross-sectional study was carried out between 2012 and 2015, on 500 men who were screened for PCa in the urology department of Imam Reza and Ghaem hospitals, Mashhad, Iran. The screening method was digital rectal examination (DRE) and serum prostate specific antigen (PSA) level.

Serum PSA was measured before obtaining biopsies by a commercial enzyme-linked immunosorbent assay (ELISA) kit (CIS Bio International, France). Then, an expert urologist performed TRUS-biopsy for all suspicious patients. Around 12-14 needle biopsies were performed for all the patients from different parts of prostate gland: six specimens from each lobe and two specimens from the apex. Each specimen was labeled and sent separately for pathologic evaluation.

### *Immunohistochemical assessment*

Paraffin blocks and microscopic slides were prepared from prostate specimens in the laboratory by hematoxylin and eosin staining. All specimens were evaluated by an expert urologic pathologist.

Specimens from 60 consecutive PCa patients, after being fixed in formalin and embedded in paraffin, were evaluated for further pathologic criteria. The pathologic examination included most prevalent grade of malignancy, microscopic Gleason score, number of biopsies with HER2 expression, the percentage of involvement in each biopsy, tumor volume (mean percentage of HER2 expression among all taken biopsy specimens), and extraprostatic invasion. Immunohistochemical staining for HER2 on paraffin-embedded sections

was also assessed using Histostain-plus kit instructions.

Expression level of HER2-neu receptor was assessed according to manufacturer's instructions (immunohistochemistry antibody, DAKO; Glostrup, Denmark), as: 0 (no or <10% HER2-neu membranous staining), 1+ (partial staining in >10% of cells), 2+ (weak to moderate complete staining in more than 10% of cells), or 3+ (strong complete staining in >10% of cells). Patients with scores 2+ and 3+ were considered positive for HER2 overexpression (9).

### *Data analysis*

All variables were separately recorded for each specimen in the questionnaire.

Patients' age was recorded as a quantitative independent continuous variable and a whole number in years. Gleason score was a quantitative discontinuous variable, registered as a whole number between 2 and 10. Serum PSA was calculated in ng/ml.

Data analysis was performed in SPSS software version 18 (IBM, Chicago, US). Group comparisons of normally distributed variables were performed using the independent-samples *t*-test. For non-normally distributed variables, Mann-Whitney or Kruskal-Wallis non-parametric tests were conducted. Data are expressed as the mean  $\pm$  standard deviation. *P* of less than 0.05 was considered as statistically significant difference.

### *Ethical considerations*

It should be noted that it is a general policy of Mashhad University of Medical Sciences affiliated hospital that all patients were asked to sign an informed consent form upon their freewill agreeing. Patients who refused to participate were not included in the present study. Also, those who pathologically ruled out to have PCa excluded from the study protocol was reviewed and approved by the ethics committee at Mashhad University of Medical Sciences (Project code: 911319).

## Results

### *Descriptive results*

Of the 500 cases underwent TRUS biopsies, 60 had microscopic PCa criteria and thus were included in the study. The mean age of these adenocarcinoma patients in our study was 68.4 years (ranging between 42-86 years).

Mean Gleason score (GS) was  $3.48 \pm 1.67$ . Grade 3 of Gleason score was the most common grade among the collected cases (38 cases representing 63.3%), while grade 5 was the least common (N=7; 11.7%). We divided Gleason score into 5 prognostic groups, which included group one with  $GS \leq 6$ , the second group with  $GS=3+4$ , the third group with  $G.S=4+3$ , the fourth group

**Table 1.** Frequency of various Gleason scores and PGG among the studied patients

PGG	Gleason score	Frequency	Percentage
I	≤6	23	38.3
II	3+4	15	25
III	4+3	13	21.7
IV	8	4	6.7
V	9 and 10	5	8.3

PGG: prognostic grade groups

with GS=8, and the fifth group with GS of 9-10 (12, 13). The most common Gleason score was related to group one, and the least common Gleason score belonged to groupFour (6.7%).

Table 1 shows the frequency of gleason scores and PCG among the patients.

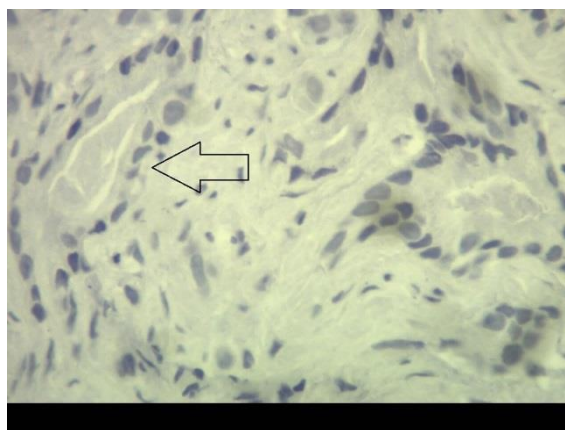
The tumor extension beyond the prostate was seen in 10 patients (16.7%). The tumor volumeranged between 0.5% and 95%.In this study, we divided the tumor volume into 2 groups of ≥30%and<30 %.Among 60 patients included in this study, 31 (51.7%) had tumor volume of less than 30% and 29 (48.3%) had tumor volume greater than 30%.Among 60studied patients, the lowest pre-biopsy PSA serum level was 5.8ng/ml and the highest was

211ng/ml. Mean PSA level among patients was 52.4±24.8 ng/ml. In this study, PSA values were divided into 3 groups of less than 4ng/ml, 4-10ng/ml, and greater than10ng/ml.None of the patients had PSA of less than four, while 15 patients (25%) hadPSA of 4-10, and 45 (75%) had PSA level of more than 10.

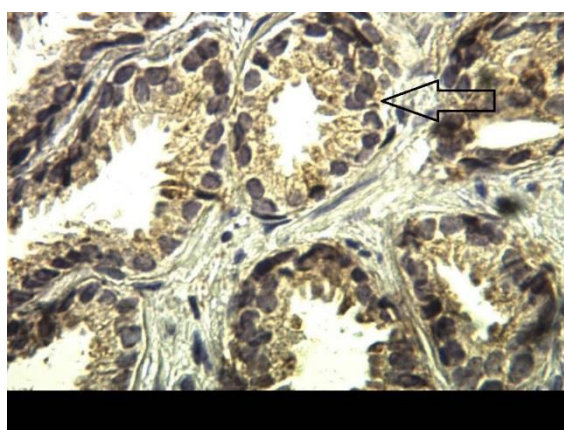
For each patient, adenocarcinoma involved biopsies were ranged from minimum of one section biopsy to maximum of 12 section biopsies. We put the number of involved biopsies in 2 groups of ≥3 (13 patients representing 21.7% patients) and <3 (47 patients representing 87.3% cases).

The patients were divided into 4 groups according to percentage of biopsies that had adenocarcinomainvolvement: 0-25% group (N=9; 15%), 25-50% group (N=11; 18.3%), 50-75% group (N=10; 16.7%), and 75-100%group (N=30; 50%).

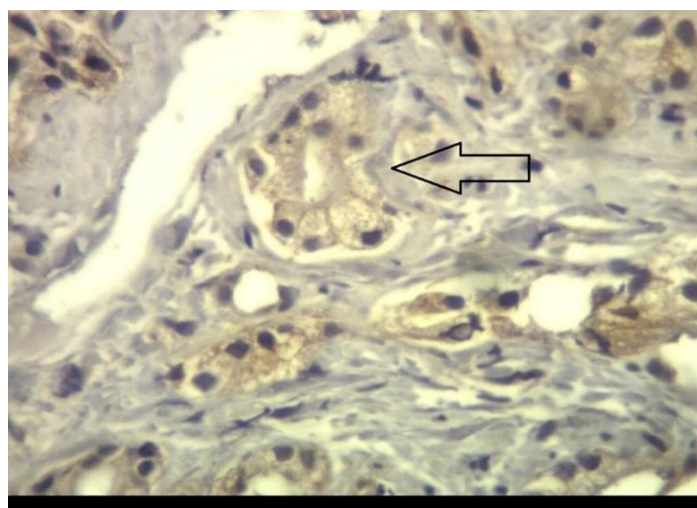
The expression of HER2 was scored between 0 and 3+ according to the Herceptintest method. Patients with scores of 2+ and 3+ were considered positive for HER2 overexpression (Figures 1-3).In this assessment, the expression



**Figure 1.** No cell membrane staining with HER2-neu(grade0); 400x



**Figure 2.** Weak membranous staining of tumor cells with HER2-neu(grade 1+);400x



**Figure 3.** Moderate membranous staining of tumor cells with HER2-neu(grade2+)

of HER2-neu was defined as either negative, weakly positive, or strongly positive. Of the 60 patients evaluated, 49 (81.7%) expressed negative HER2-neu expression and 11 patients (18.3%) had weakly positive expression. None of the patients had strongly positive HER2-neu expression (Table 2 and table 3 show the frequency of Her2-neu in patients and relationship between Her2-neu and tumor volume in patients, respectively.)

### Analytical results

A significant association between HER2-neu

incidence and tumor volume was found in our study ( $P=0.002$ ). All patients with positive HER2-neu expression, had tumor volume of more than 30%. However, the expression of HER2-neu was not significantly associated with serum PSA level ( $P=0.26$ ). No significant relationship was detected between HER2-neu expression and Gleason score ( $P=0.70$ ).

There was no significant relationship between the incidence of HER2-neu and other parameters, including the number of involved biopsies, percentage of biopsy involvement, and extraprostatic invasion of tumor (Table 4).

**Table 2.** Frequency of HER2-neu immunohistochemical staining in participants

HER2-neu expression	Degree of staining	Frequency	Percentage
negative	0	35	58.4
	+1	14	23.3
weakly positive	+2	11	18.3
strongly positive	+3	0	0

HER2: human epidermal growth factor receptor 2

**Table 3.** Relationship between HER2-neu expression and tumor volume in participants

Tumor volume	HER2-neu expression		Total
	Negative	Weakly positive	
≥30%	30	1	31
<30%	19	10	29

HER2: human epidermal growth factor receptor 2

**Table 4.** Association of HER-2 Expression and other Clinical and pathological Findings in specimen of participants

variables	values	HER-2 (+)	HER-2 (-)	P
Serum PSA level (Prior to biopsy taking)	<4	0 (0 %)	0	0.26
	4-10	15 (25 %)	1	
	>10	45 (75 %)	10	
Tumor volume	≥30 %	31 (51.7%)	1	0.002
	<30%	29 (48.3%)	10	
Gleason score	≥6	23 (38.3%)	3	0.70
	3+4	15 (25%)	3	
	4+3	13 (21.7%)	4	
	8	4 (6.7%)	1	
Extra-prostate extension	9 and 10	5 (8.3%)	0	0.45
	positive	10 (16.7%)	1	
	negative	50 (83.3%)	10	
Adenocarcinoma involved biopsies	0-25%	9 (15%)	1	0.59
	25-50%	11 (18.3%)	0	
	50-75%	10 (16.7%)	0	
	75-100%	30 (50%)	10	

HER2: human epidermal growth factor receptor 2

## Discussion

Prostate carcinoma is the most common internal malignancy among the American men and is responsible for 10% of deaths due to cancer in this population. Prostate cancer is the secondmost common lethal cancer in men (3). Incidence of prostatic carcinoma in Iran is very low compared with western countries, which could be due to the younger population and also the lack of national screening programs in Iran (14).

HER2 is one of the human EGFR (HER/EGFR/ERBB) family, which is located on chromosome 17 (12). In prostate carcinoma, steroid hormones and growth factors play a

regulating role in cell proliferation and HER2-neu is associated with the activation of the androgenic receptor pathways. Therefore, it might be a survival factor for hormone-resistant prostate cancers (16).

Some previous studies have reported that HER2-neu expression measured by immunohistochemical staining was associated with less survival rates. Therefore, HER2-neu inhibition may be a therapeutic strategy for hormonal-resistant prostate cancer. But unfortunately, its effects have not yet been proven clinically (16).

HER2-neu overexpression affects the growth and prognosis of some tumors (12). There is

HER2-neu overexpression in about 30% of patients with breast cancer, which may predict the response of cancers to the anti-HER2-neu monoclonal antibody therapy (trastuzumab). Moreover, HER2-neu expression is different in PCa due to its tendency to increase expression in both advanced and non-androgen-dependent prostate carcinomas(12).

In our study, the expression of HER2-neu was investigated in prostate carcinoma. Based on our results, 18.3% of the 60 patients had weakly positive HER2-neu expression and the others were negative. Results of the present study were in agreement with previous studies, in which HER2 amplification was reported in 13.3% of 150 patients using the same staining method (16), whereas another analysis reported HER2 amplification as high as 72.97% of 59 patients (12).

In the current study, the expression of HER2-neu was not significantly associated with serum PSA level of the patients, which is similar to the results obtained in a series of studies (12-19). However, some studies have also reported a significant association between HER2-neu expression and serum PSA levels(20).

Like most previous studies(16, 18, 20, 21), our study did not find any significant relationship between HER2-neu expression and Gleason score. In contrast, there was a significant association between these two factors in a study by Zhangyi-fen et al. (19). These conflicting results may be due to variations in the sample size and methodology, staining and fixation protocols, as well as different grading systems or genetic heterogeneity.

We also did not find any statistical relationship between the expression of HER2-neu and Gleason grade, which is consistent with the findings of Duygn et al. who could not demonstrate any relationship between these two factors(17).

There was no significant relationship between the incidence of HER2-neu and other parameters, including the number of involved biopsies, the highest percentage of biopsy involvement, and extra prostatic invasion of tumor, which could not be compared due to the lack of similar studies in the literature. A significant association between HER2-neu incidence and tumor volume was found in our study.

## Conclusion

Among several variables evaluated in our study, only tumor volume had significant statistical association with the expression of HER2-neu. Tumor volume is an important prognostic factor that is associated with lymph node involvement, capsular invasion and seminal vesicle involvement in PCa.

Our findings highlight the need for a standardized and organ-specific immunohistochemical methodology to determine HER2-neu status in patients with prostate carcinoma. We also suggest further studies in order to evaluate HER2-neu gene amplification with these prognostic factors in radical prostatectomy specimens.

## Acknowledgements

None.

## Conflicts of Interest

None.

## References

1. Kolahdoozan S, Sadjadi A, Radmard AR, Khademi H. Five common cancers in Iran. Arch Iran Med. 2010; 13(2):143-6.
2. Tao Z, Shi A, Wang K, Zhang W. Epidemiology of prostate cancer: current status. Eur Rev Med Pharmacol Sci. 2015; 19(5):805-12.
3. Bashir MN. Epidemiology of prostate cancer. Asian Pac J Cancer Prev. 2015; 16(13):5137-41.
4. Pernar CH, Ebot EM, Wilson KM, Mucci LA. The epidemiology of prostate cancer. Cold Spring Harb Perspect Med. 2018; 8(12):a030361.
5. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J. 2000; 19(13):3159-67.
6. Yeh S, Lin HK, Kang HY, Thin TH, Lin MF, Chang C. From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells. Proc Natl Acad Sci. 1999; 96(10):5458-63.
7. Edwards J, Mukherjee R, Munro AF, Wells AC, Almushat A, Bartlett JM. HER2 and COX2 expression in human prostate cancer. Eur J Cancer. 2004; 40(1):50-5.
8. Fonseca GN, Srougi M, Leite KR, Nesrallah LJ, Ortiz V. The role of HER2/neu, BCL2, p53 genes and proliferating cell nuclear protein as molecular prognostic parameters in localized prostate carcinoma. Sao Paulo Med J. 2004; 122(3):124-7.
9. Lara PN, Meyers FJ, Gray CR, Edwards RG, Gumerlock PH, Kauderer C, et al. HER-2/neu is overexpressed infrequently in patients with prostate carcinoma. Results from the California Cancer Consortium Screening Trial. Cancer. 2002; 94(10):2584-9.
10. Carles J, Lloreta J, Salido M, Font A, Suarez M, Baena V, et al. Her-2/neu expression in prostate cancer: a dynamic process? Clin Cancer Res. 2004; 10(14):4742-5.
11. Nishio Y, Yamada Y, Kokubo H, Nakamura K, Aoki S, Taki T, et al. Prognostic significance of immunohistochemical expression of the HER-2/neu oncoprotein in bone metastatic prostate cancer. Urology. 2006; 68(1):110-5.
12. Rubin MA, Girelli G, Demichelis F. Genomic correlates to the newly proposed grading prognostic groups for prostate cancer. Eur Urol. 2016;

- 69(4):557-60.
13. Sauter G, Steurer S, Clauditz TS, Krech T, Wittmer C, Lutz F, et al. Clinical utility of quantitative Gleason grading in prostate biopsies and prostatectomy specimens. *Eur Urol*. 2016; 69(4):592-8.
  14. Sadjadi A, Nooraie M, Ghorbani A, Alimohammadian M, Zahedi MJ, Darvish-Moghadam S, et al. The incidence of prostate cancer in Iran: results of a population-based cancer registry. *Arch Iran Med*. 2007; 10(4):481-5.
  15. Mofid B, Jalali Nodushan M, Rakhsha A, Zeinali L, Mirzaei H. Relation between HER-2 gene expression and Gleason score in patients with prostate cancer. *Urol J*. 2009; 4(2):101-4.
  16. Siampanopoulou M, Galaktidou G, Dimasis N, Gotzamani-Psarrakou A. Profiling serum HER-2/NEU in prostate cancer. *Hippokratia*. 2013; 17(2):108-12.
  17. Baltaci S. Her-2/neu oncogene expression in prostate carcinoma: evaluation of gene amplification by FISH method. *Turkish J Pathol*. 2008; 24(2):76-83.
  18. Fantinato AP, Tobias-Machado M, Fonseca F, Pinto JL, Wroclawski ML, Wroclawski E, et al. HER2/neu expression by reverse transcriptase-polymerase chain reaction in the peripheral blood of prostate cancer patients. *Tumori*. 2007; 93(5):467-72.
  19. Zhang YF, Guan YB, Yang B, Wu HY, Dai YT, Zhang SJ, et al. Prognostic value of Her-2/neu and clinicopathologic factors for evaluating progression and disease-specific death in Chinese men with prostate cancer. *Chin Med J*. 2011; 124(24):4345-9.
  20. Shuch B, Mikhail M, Satagopan J, Lee P, Yee H, Chang C, et al. Racial disparity of epidermal growth factor receptor expression in prostate cancer. *J Clin Oncol*. 2004; 22(23):4725-9.
  21. Mofid B, Nadoushan MJ, Rakhsha A, Mirzaei H, Zeinali L. Epidermal growth factor receptor gene in prostate cancer after radical prostatectomy. *Iran J Cancer Prev*. 2010; 3(4):174-7.