RESEARCH ARTICLE

# Overexpression of HER-2/neu in Patients with Prostatic Adenocarcinoma

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## Abstract

**Background: Prostatic adenocarcinoma is one of the main causes of cancer death, and its timely diagnosis and preventing its progression dramatically helps improve life indexes. Given the high disease recurrence rate, today, research is more inclined toward exploring causes of recurrence and development, and innovation of modern treatment methods. Several studies have explored over-expression of human epidermal growth factor receptor 2 (HER-2/neu) in prostatic cancer so far, with different results. Thus, it was decided to investigate HER-2/neu overexpression in patients with prostatic adenocarcinoma in Iran. Materials and Methods: A sample size of 40 patients with prostate cancer entered the study, using a cross-sectional, non-randomized sampling method. Parameters studied included patient age at surgery, Gleason score, serum prostatic specific antigen (PSA) before surgery, and positive sample rate after immunohistochemical staining to investigate HER-2/neu overexpression. Results: In terms of HER-2/neu receptor staining rate, of 40 slides, 16 (40%) scored 0, 13 (32.5%) 1+, 7 (17.5%) 2+, and 4 (10%) 3+. In total 27.5% of slides showed HER-2/neu overexpression. In terms of age, an inverse correlation was found (-0.181), but without significance (p=0.263). In terms of serum PSA, the correlation coefficient was 0.449 (p=0.004). With respect to Gleason score, the coefficient was 0.190 (p=0.240). Conclusions: In this study, HER-2/neu overexpression occurred in 27.5% of prostate cancer cases, which is a relatively high figure, compared to similar studies elsewhere. While, we failed to reveal any relationship between HER-2/neu expression status with progression and prognosis of disease, it was demonstrated that the serum PSA level was significantly higher in cases with increased receptor expression.**

**Keywords:** Prostate - adenocarcinoma - HER-2/neu - prostate specific antigen

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## Introduction

Prostate adenocarcinoma is the most common malignant prostatic neoplasia, and a leading cancer among men, claiming a high percentage of mortality (Nath et al., 2012; Muhammadnejad et al., 2013). In 2012, it was the leading new cancer case among men in the United States, accounting for 29% of all new cases of cancer in men, equivalent to sum total of lung and bronchus, colorectal and bladder cancers. In terms of cancer deaths, next to lung and bronchus cancers, it is the second leading cancer among men. The prevalence of this cancer is lower in Asian countries. Yet, in recent years, its incidence has been increasing, compared to Western countries (Hsing et al., 2000; Siegel et al., 2012).

In early stages of the disease, treatment is local, including surgery (radical prostatectomy) and radiotherapy, even though the disease may progress in up to 40% after surgery, causing treatment-resistant cases. According to modern studies, this progress is usually attributed to early and microscopic spread to cancer cells, which is not traceable with current diagnostic techniques. Much effort has been made to identify causes of advancing cancer and patients susceptible to advanced prostate cancer (Neto et al., 2010). Currently, research is sloping toward exploring more specific biomarkers of invasive prostate cancer, so that, they can be considered definitely involved in prognosis of this cancer, and treatment can be based on their existence. One of these biomarkers is growth factors and their receptors, and HER-2/neu tops the list (Baek et al., 2012).

HER-2/neu proto-oncogene is located on chromosome 17q, and encodes a 185 KDa trans-membrane glycoprotein receptor, known as p185 neu, HER-2, and erbB-2. HER-2 is derived from Human Epidermal growth factor Receptor, due to its similarity with Epidermal Growth Factor Receptor (EGFR) (Liu et al., 2012). This receptor is involved in various biological processes such as cell proliferation, differentiation, migration, and apoptosis. There is low expression of HER-2 in normal epithelial cells, including prostate epithelial cells (Montironi et al., 2006; Ali Nahit Sendur et al., 2012; Carrion et al., 2012).

Occasionally, this oncogene is involved in amplification, resulting in overexpression of HER-2/neu receptor at cell

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level, which has been demonstrated in almost 30% of breast adenocarcinomas, and is also more or less seen in ovarian, stomach, uterus, and prostate cancers and so forth (Begnami et al., 2011; Ali Nahit Sendur et al., 2012). In recent years, many studies have been conducted on HER-2/neu overexpression in prostate adenocarcinoma and its effect on characteristics of cancer, and conflicting results have been found on involvement of HER-2/neu overexpression in prostate cancer and driving it toward resistance to normal treatments, and subsequent reduced survival and worsening of disease prognosis (Gates et al., 2009).

A strong relationship between HER-2/neu overexpression and greater mortality and recurrence rates in prostate cancer patients was describe, and concluded that overexpression of this receptor should be considered as a definite criterion in prostate adenocarcinoma prognosis (Neto et al., 2010). In a study researchers found that pretreatment serum HER-2neu may has a better prediction for recurrence of metastatic prostate carcinoma than immunohistochemical overexpression (Tambo et al., 2009). Thus, it was decided to determine the expression of this gene in patients with prostate cancer at this center to know its status.

## Materials and Methods

*Study population*

This study was conducted on men with prostate adenocarcinoma, and necessary samples were collected from pathology archives of radical prostatectomy cases with diagnosis of adenocarcinoma in Shahid Sadoughi, Mortaz and Mojibian-Yazd hospitals. This cross-sectional study was conducted in observational descriptive method. Based on previous studies, with 95% confidence and relative prevalence of 30% to 40% of HER-2/neu overexpression, and minimum difference of 15%, using the equation below, sample size was estimated at 40 patients. The following variables were studied: Patient's age at biopsy, microscopic Gleason score, pre-biopsy serum PSA level, and positivity score after immunohistochemical staining for assessment of HER-2/neu overexpression. Patient's age was extracted as a quantitative independent continuous variable and a whole number in years. Gleason score of each sample was a quantitative discontinuous variable, registered as a whole number between 2 and 10. Patients' serum PSA was calculated in nanogram per milliliter (ng/ml). Expression level of HER-2/neu receptor was assessed according to manufacturer's instructions (immunohistochemistry antibody, DAKO; Glostrup, Denmark), as: 0 (No HER-2/neu membrane staining, or observed in less than 10% of cells), +1(Partial staining in more than 10% of cells), +2 (Weak to moderate complete staining in more than 10% of cells), or +3(Strong complete staining in more than 10% of cells). All these variables were separately prepared and recorded for each sample in the questionnaire.

*Methods*

First, slides stained by hematoxylin and eosin (H&E) method were procured from prostate adenocarcinoma blocks in the archives that were formalin-fixed and paraffin-embedded, and all were examined separately by two pathologists. The required 40 samples were extracted. Samples' Gleason grading was performed and recorded by two pathologists, concurrently, and without one another's knowledge. Patients' age and serum PSA level were also extracted from pathology reports.

Because it is less costly compared to other methods, and relatively easier and less time-consuming, immunohistochemistry is the most common technique for evaluation of HER-2/neu overexpression. Staining of slides was carried out according to DAKO Company instructions. Every slide was stained in this way, and made available to the pathologists for scoring.

All 40 slides were examined by two pathologists, without knowledge of other variables associated with samples, and according to DAKO Company instructions, so that, based on membrane staining intensity, which is indicative of expression of HER-2/neu receptor, each sample received scores equivalent to 0, 1+, 2+, or 3+. All score 2+ specimens were examined by fluorescent in situ hybridization (FISH) method for definition of amplification of HER-2/neu receptor (FISH ratio > 2 considered as positive status).

In statistical analysis, expressions scores 2+, and 3+, were considered overexpression of HER-2/neu, based on DAKO scoring criterion.

*Statistical analysis*

After quality control, data were entered into SPSS software version 16 for windows (IBM Inc., NY, US) and the required tables and indicators were prepared, using the same software, supervised by the project statistician. Statistical tests t-test and Pearson correlation coefficient were used to determine relationships and make comparison.

*Ethical considerations*

It should be noted that, it is a general policy of Shahid Sadoughi University of Medical Sciences affiliated hospitals that all patients are asked to sign an informed consent form upon their freewill agreeing that their medical records be used for research purposes. Patients refusing to consent were not included in the present study. Study protocol was reviewed and approved by medical ethics committee at Shahid Sadoughi University of medical Sciences.

## Results

First, preliminary assessment of age, serum PSA level, and Gleason score was separately performed. In terms of age at surgery and taking samples, study subjects were aged 52 to 90 years, with mean age of 74.2±9.2 years. Patients' mean serum PSA at surgery, extracted from patients' pathology report, was 48.4±28.8 ng/ml (range 8 to 100). Results of Gleason score were mean±SD 6.13±1.77 (range: 3 to 10). IHC staining to assess overexpression of HER-2/neu receptor showed the following results: 16 slides (40%) had no sign of expression of this receptor, and thus scored 0. In 13 cases (32.5%) expression of

receptor was about 1+, in 7 cases (17.5%) expression of receptor was 2+, and finally, in 4 cases (10%) expression of HER-2/neu was about 3+, as reported by pathologists.

As discussed earlier, 2+ and 3+ scores were considered overexpression in HER-2/neu receptor. According to this classification, 29 cases (72.5%) showed no overexpression of this receptor, and overexpression (the main objective of this study) was observed in 11 cases (27.5%) (Table 1).

Next, using T-test, mean age, serum PSA, and Gleason score, in two groups, data were separately compared based on HER-2/neu overexpression (Figure 1 and Table 2).

According to (Table 2), mean age of the group with no overexpression was found 75.6±8.3 years, and mean age of the group with overexpression was 70.5±10.7 years. Given p=0.122, the difference in mean age between the two groups was insignificant. Mean PSA level in the group without overexpression was 41.9±27.7 ng/ml, and mean PSA in the group with overexpression was 65.4±26.6 ng/ml. p=0.019 is indicative of a significant difference between the two groups. Mean Gleason score in the first group was 6.1±1.6, and in the second group, mean score was 6.4±2.1. So p=0.606 is indicative of an insignificant difference between the two groups.

**Table 1. Scoring Results of Slides Stained to Assess HER-2/neu Receptor with DAKO Criterion**

| Expression level | Quantity | (%) | Quantity | (%) |
|---|---|---|---|---|
| 0 | 16 | 40 | 29 | 72.5 |
| + | 13 | 32.5 | | |
| ++ | 7 | 17.5 | 11 | 27.5 |
| +++ | 4 | 10 | | |
| Total | 40 | 100 | 40 | 100 |

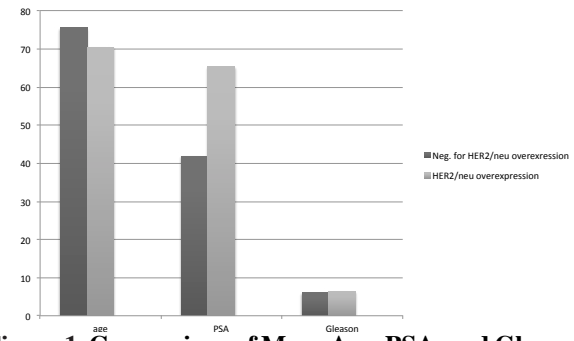


**Figure 1. Comparison of Mean Age, PSA, and Gleason Score Based on Overexpression of HER-2/neu**

**Table 2. Comparison of Mean Age, PSA, Gleason Score and Pearson Correlation Coefficient According to Overexpression of HER-2/neu in Patients with Prostate Cancer**

| | HER-2/neu overexpression | Mean | p value | **HER-2/neu score |
|---|---|---|---|---|
| Age (years) | - | 75.6±8.3 | 0.122 | -0.181 |
| | + | 70.5±10.7 | | |
| *PSA (ng/ml) | - | 41.9±27.7 | 0.019 | 0.449 |
| | + | 65.4±26.6 | | |
| Gleason score | - | 6.1±1.6 | 0.606 | 0.190 |
| | + | 6.4±2.1 | | |

*According to T-test, there was an insignificant difference between the two groups in mean age and Gleason score, but with respect to PSA, the difference was significant ($p$=0.01); **Pearson correlation coefficient showed insignificant correlation of variations in age and Gleason score with variations in HER-2/neu expression. However, HER-2/neu overexpression significantly increased with increasing PSA level (p=0.004)

In the next step, Pearson correlation coefficient was used to assess the relationship of increased age, PSA and Gleason score with overexpression rate of HER-2/neu receptor (Table 2).

In relation to age, correlation coefficient was found -0.181, with p=0.263, indicating an inverse relationship between age and rate of receptor expression, which was insignificant. Correlation coefficient was found 0.449 for serum PSA level, and p=0.004 indicated a significant correlation between increased PSA level and increased HER-2/neu expression. For Gleason score, correlation coefficient was found 0.190, and p=0.240, indicating an insignificant correlation.

## Discussion

In the present study, overexpression of HER-2/neu receptor using IHC and FISH methods occurred in 27.5% of patients, which is higher than that in a study by United States National Cancer Institute in 2002. They used IHC technique, and observed overexpression in only 5 out of 62 patients: one scored 3+, and the remaining 4 scored 2+. They concluded that HER-2/neu overexpression had occurred in only 8% of patients, and unlike breast cancer, this level was insignificant (Lara et al., 2002) .Other studies also contradicted results obtained in the present study including that overexpression occurred in only 1.75% of patients (Calvo et al., 2003). Of course (Siampanopoulou et al., 2013) had shown in their study that higher serum levels of HER-2/neu had been associated with higher rate of recurrence and metastasis. Furthermore they emphasized that, there was a correlation between HER-2/neu levels and poor prognosis in prostate carcinoma (Siampanopoulou et al., 2013).

According to this study and compared to the study by United States Cancer Institute in 2002, no significant relationship was found between age and Gleason score with HER-2/neu expression (Lara et al., 2002). Although in the present study, expression of this receptor was higher in younger ages, which can somewhat indicate more intense involvement in the presence of HER-2/neu overexpression. However, based on the present study results, overexpression of HER-2/neu cannot be considered a definite factor in determining prognosis of the disease. This finding disagrees with the results of some other studies. A study, conducted in a hospital in Shanghai-China in 2007, showed a significant relationship between expression of HER-2/neu and Gleason score, such that, receptor expression increased with Gleason scores above 7 (Bai et al., 2007). Other researchers in 2010, described that there was a consistent relation between HER-2/ neu and Gleason score which in patients with score less than 7 the mortality and morbidity rate was higher than patients with higher Gleason score and they recommended more clinical trial studies to investigate the association of Her-2/neu overexpression and worsening of the outcome (Neto et al., 2010).

The present study is different, in that, not only a significant difference was found in serum PSA level between the two groups based on HER-2/neu overexpression, but also Pearson correlation coefficient

showed that serum PSA level significantly increased with HER-2/neu overexpression. This relationship has not been cited in similar studies. However, in studies conducted to investigate the effect of serum PSA on intensity and prognosis of diseases, it is concluded that its serial assessment and doubling time play a role in determining prognosis of disease, and assessment of this marker only once, cannot provide the basis for decision making (Loeb et al., 2008;Vickers et al., 2012) .

Although (Di Lorenzo et al., 2004) in their study which was done on androgen dependent and advanced androgen independent prostate cancer patients revealed that overexpression of HER-2/neu had an inverse effect on prognosis and recurrence after its endocrine treatment. Shariat et al. (2007) had shown in their study that preoperative measurement of plasma HER -2/neu and EGFR had useful predictive value for tumor and PSA progression which high plasma HER-2/neu level with low EGFR rate associated with higher levels of HER-2/neu expression. Increased initial PSA levels could worsen the prognosis of prostatic carcinoma ,with early bone and distant metastasis (Siampanopoulou et al., 2013), and also overexpression of Her2/neu could lead to progression of prostate adenocarcinoma ,so our findings were in concordance with other findings , that in our study patients with elevated PSA levels had more over expression of HER-2/neu than patients with lower levels of PSA.

The role of HER-2/neu receptor in pathogenesis and progress of prostate cancer is still unknown. Currently, given modern treatments that directly target this receptor, determining its level of expression has become increasingly important. In this process, different expression assessment methods are used that can be a factor in producing different and conflicting results in similar studies. In the present study, IHC technique was used to investigate samples. Although this technique is more commonly used, easier and less expensive, it is also less accurate than other methods, which can account for potential flaws in this study. Assessment of HER-2/neu expression in patients' serum, requires an accurate, but more expensive FISH technique, and method of assessment of gene level associated with this receptor, are among other methods for studying this receptor, and are recommended for use in future studies.

In the present study, patients' age showed an inverse and insignificant relationship with expression of HER-2/neu receptor. In future studies with larger sample size and a significant relationship between younger age and higher expression of this receptor, role of this receptor in worsening prognosis of prostate cancer can be spoken of with greater certainty.

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