Original Article

Expression And Scoring Of HER2/Neu in Variants of Prostate Adenocarcinoma

Santosh Kumar Sidhwani, Madeeha Sadiq, Mubina Lakhani, Sumayyah Shawana, Raffia Siddiqui, Sobia Hassan

ABSTRACT

Objective: To evaluate the immunohistochemical expression and scoring of HER2/neu in different variants of adenocarcinoma of prostate and to compared the association of HER2/neu expression with biological behavior and risk factors of prostate adenocarcinoma.

Study Design and Setting: Cross sectional study in which all clinically suspected prostate adenocarcinoma cases received at the laboratory Saddar Karachi during the years 2015 and 2016 were evaluated for morphological features of adenocarcinoma.

Methodology: This cross sectional study was carried out using prostate biopsies of clinically suspected prostate adenocarcinoma. The diagnosis of adenocarcinoma was confirmed and histological characterization was done by evaluating the morphological features. The tumors were graded according to the revised 2015 Gleason's grouping. Immunohistochemical analysis for HER2/neu expression was performed in the most representative tumor block. SPSS version 22 was used for data analysis. Mean frequency and percentages were calculated for quantitative variables, whereas chi-square test and Fisher's Exact Test were applied for qualitative variables. P-value of < 0.05 was considered as significant.

Results: Out of 77 biopsies only one showed moderate HER2/neu expression. Positive HER2/neu was acinar variant. No significant statistical association was observed between expression of HER2/neu and prostate cancer variants. The positive case had age more than 60 years with moderately increased serum PSA levels and was aggressive in nature at the time of diagnosis.

Conclusion: It was concluded from the study that HER2/neu was rarely expressed in prostate adenocarcinoma.

Keywords: Adenocarcinoma Prostate, Acinar, Ductal, Gleason Groups, PSA levels, Variants, HER2/neu.

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INTRODUCTION:

Prostate cancer is second most prevalent cancer affecting males worldwide.[1] About every 7th man in his lifetime is diagnosed with the prostate cancer.[2] According to GLOBOCON 2018, the prevalence of prostate adenocarcinoma is 7.1% and about 1.3 million of new cases have been reported in same year with a death rate of 24.1 per 100,000 males.[3] Prostate cancer is known to affect the older age group with a mean age of 65 years and above.[4] Nations with high prevalence are New Zealand, Australia and South Africa where as lower rates have been seen in less developed countries.[5]

In Pakistan, an increasing trend of prostate cancer has been recorded with a prevalence of 5.3% per 100,000 in recent years.[6] Till now, the established causes of the prostate adenocarcinoma remained obscure, though multiple risk factors have been described which include hormones, age, family history, race, genetics, androgen receptors and many others.[7] The pathogenesis of prostate cancer mainly depends on cell growth and differentiation affected by the genes and androgens and their binding to their receptors.[8]

Diagnosis of prostate cancer mainly depends on history, clinical examination like DRE (digital rectal examination) sign and symptoms, radiological examination and PSA levels.[9] But still, the gold standard is biopsy and histopathological examination. Histologically, prostate adenocarcinoma exhibits neoplastic glands lined by a single layer of cuboidal cells. While the immunohistochemistry helps in diagnosis of the many cancers including the prostate adenocarcinoma.[10] According to recent studies, there are several immune markers expressed by prostatic tissue and

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one of them is HER2/neu.[11] It has been demonstrated that HER2/neu has been expressed in high pathological stage and highly aggressive prostate adenocarcinoma.[12]

HER2/neu expression could be targeted for the novel therapeutics intervention in prostate adenocarcinoma as implicated in other tumors like breast cancer. Several studies have postulated that HER2/neu expression in prostate cancer could be responsible for differentiation, angiogenesis and alteration in cellular proliferation migration, survival and tumor formation. In many tumors, HER2/neu is proved to be good prognostic marker thereby providing a vital therapeutic option.[13]

Therefore, the present study was aimed to evaluate the immunohistochemical expression of HER2/neu in different variants of prostate adenocarcinoma and to compare its expression with the different clinicopathological parameters of prostate cancer.

METHODOLOGY:

This study was conducted after approval from ethical review committee of Ziauddin University (Ref Number. 0110306SKPATH). All clinically suspected prostate adenocarcinoma cases received at The Laboratory Saddar Karachi during the years 2015 and 2016 were evaluated for morphological features of adenocarcinoma. We excluded the cases with benign prostatic hyperplasia, secondary cancer or metastatic to prostate, cases with other benign prostatic lesion, prostatic intraepithelial neoplasm and cases on anti-androgen therapy.

After confirming the diagnosis, 77 cases were randomly selected following informed consent along with pertinent clinical history and PSA levels. Tumor was classified into ductal and acinar vaiants while the representative Gleason's group was assigned by examining multiple levels of Hematoxylin and Eosin stained sections by the consultant histopathologist. The most representative tumor block bearing abundant tumor volume was selected for immunohisto-chemistry. The study was conducted following ethical approval from ethics review comity (ERC) of Ziauddin University Karachi. We performed immunohistochemistry by the method described by Signoretti et al.[14] The paraffin embedded, formalin fixed prostatic tissue blocks were selected and cut into 3µm sections and dipped into water. After transferring the tissue to glass slides these sections were treated with citrate buffer (0.1mol/L) for 45 minutes in microwave for antigen retrieval. A mouse monoclonal antibody (HER2/neu, cell marque) was prepared at dilution of 1:50ml. Tissue sections were then treated with prepared antibody for 45 minutes followed by rinsing with buffer, followed by separately developed enzyme activity. These sections were then treated with graded alcohol and counter stained with hematoxylin for 1 minute followed by distilled water rinse and later dried at room temperature. Cover slip was applied on the slide using mounting media. Known HER2/neu positive breast adenocarcinoma slides were used as positive controls while slides incubated without primary antibody in tris buffer (TBS) were used as negative controls. These slides with immunohistochemical sections were then evaluated for stating pattern of HER2/neu by the same panel of histopathologists. The tissue sections were examined in light microscopy and sections with dark brown cytoplasmic staining were considered as positive. Statistical analysis was performed using SPSS version 22 (SPSS Inc., Chicago, USA). The association of HER2/neu expression with clinicopathological parameter, which includes; age, tumor grade and PSA levels were assessed by Chi-square test. P value less than 0.05 was considered as significance.

Table 1: Expression of HER2/neu in study subjects

HER2/neu	Prostate Adenocarcinoma
Positive	1
Negative	76
Total	77

Table 2: Statistical estimates of immunohistochemical expression of HER2/neu in different variants of prostate adenocarcinoma

Tumor Variants	HER2/neu Positive	HER2/neu Negative	P value
Acinar N=71	01	70	0.78*
Ductal N=6	00	06	

Table 3: Correlation of tumor variants with different risk factors of prostate adenocarcinoma

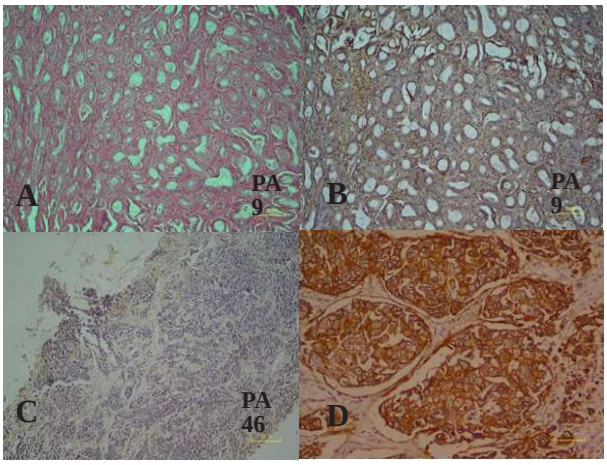
Tumor variant	Age (years)			Family history			PSA level (ng/ml)				Gleason's Group			
	≤ 60	> 60	P-value[1]	Sporadic	Familial	p-value[2]	> 4	≥ 20	> 50	P-value[3]	Mild Risk[a]	Intermediate Risk[b]	High Risk[c]	p-value[4]
Acinar n=71	12	59	0.34*	62	9	0.46*	67	4	0	0.71*	11	18	42	0.32[d]
Ductal n=6	0	6		6	0		6	0	0		0	3	3	

*Fisher's Exact Test, p-value for comparison of HER2/neu with [1]age and [2]family history
?Gleason's Group 1, ?Gleason's Group 2 and 3, ?Gleason's Group 4 and 5, *Fisher's Exact Test, **?**Pearson Chi Squere test, p-value for comparison of HER2/neu with [3]PSA level and 4Gleason's group.

RESULTS:

According to histopathological findings, the prostate cancer were differentiated into acinar (71 cases) and ductal (6 cases) variants. The immunohistochemical expression of HER2/new was evaluated in all 77 biopsies and we determined only one case that showed the HER2/neu expression (Table 1). Positive HER2/neu tumor showed acinar morphology (Figure 4.1). This has an insignificant statistical association with prostate cancer variants (Table 2)The strength of protein expression was observed 2 on scale of 3. The statistical comparison of variants of prostate adenocarcinoma with age revealed that most of the cases were aged 60 years and above at time of diagnosis. Mean age of patients in the present study was observed to be 68.7±7.9 years. Moreover, majority of the cases presented with aggressive biological behavior with high PSA levels. Unfortunately, no statistical correlation association was seen between variants of prostate adenocarcinoma and HER2/neu and these parameters. (Table 3)

Figure 1: Microscopic picture of PA 9 showing (A) H & E of neoplastic glands with variable sizes (B) moderate immuno-histochemical expression of HER2/neu in atypical glands (C) PA 46 showing HER2/neu negative with neoplastic glands that are undifferentiatedand (D) Membranous expression of HER2/neu membrane in control (Breast Adenocarcinoma). (H & E and IHC; 20x magnification



DISCUSSION:

Globally in men, prostate adenocarcinoma is found to be the second most common killer.[15] There is a rise in incidences of this specific type of tumor over the last few decades which makes therapeutic intervention and diagnosis challengeable.[12] A few previous studies demonstrated up to 53% of expression of HER2/neu in prostate adenocarcinoma while some studies failed to find any expression in their respective studies.[16] Therapeutic and prognostic significance of HER2/neu has been established in many cancers such as breast adenocarcinoma.[17] The present study however failed to generate any significant relation between prostate adenocarcinoma and expression of HER2/neu apart from one positive case of acinar variants. These findings are in line with other several studies that showed rare or no expression of HER2/neu in adenocarcinoma prostate.[18,19] These differences in present study may have been attributed due to different antibodies or different technical skills used. Else genetic variation, demographic profile and exposure to different risk factors, fixation and processing of tissue may also affect the levels of immune staining.[20] Many population based studies with moderate and high prevalence of prostate cancer suggested advancing age as a major risk factor for prostate adenocarcinoma with mean age of 65 or above, also showing that after seventies the risk is increased by two folds.[21] The mean age of prostate adenocarcinoma in the present study was found to be 68.7±7.9 years. These findings are comparable with previous studies done on including different populations.[22,23] The cases recruited in the present study are hospital based due to which the conclusion drawn on advancing age and risk of prostate adenocarcinoma cannot be generalized on population.

Two variants of prostate adenocarcinoma acinar and ductal were observed in the present study. The predominant variant was acinar with a frequency of 92.2% followed by ductal with 7.8% of patients. These frequencies are in accordance with past studies.[24,25] However, no rare morphological variants were seen due to the limited sample size. The HER2/neu positive case had morphological features consistent with the acinar variant so it was characterized accordingly. This showed an insignificant statistical association (p=0.078) with different pathological parameters. Low sensitivity of immunohistochemistry may be the reason behind the absent expression of HER2/neu in other variants.[26,27] However, FISH, PCR, gene amplification may show more reliable results due to their high sensitivity. Our results may be explained on the basis of observation of only two variants of prostate cancer in our sample.

In this study, only the acinar (mean age 68 years) and ductal entities (mean age 71 years) of prostate cancer were observed. These results showed that these two entities follow the pattern of advancing age and these findings are in agreement with previous studies.[28] A possible explanation is the late presentation of symptoms. It can by hypothesized that some factors like putative agents and formation of free radicals related to aging may contribute to causation of cancers in conjunction with other environmental factors such as sedentary lifestyle, family history and dietary habits. However more studies on large number of these entities are needed before any definite conclusion may be drawn regarding age and prostate cancer.[29]

The preoperative PSA levels of both groups were similar on comparing the pathological parameters. It was postulated that there is high risk of developing the prostate cancer before the age of 60 years in individuals having a strong family history. Such patients may be benefited from regular PSA screening compared to general population. PSA levels

and Gleason score and tumor stage are considered as best prognostic factors for prostate adenocarcinoma progression. Additionally, new Gleason grouping system provides a better comparison and better assessment of invasion, growth pattern and morphological differentiation. Furthermore, as recommended by new Gleason grouping system, prostate adenocarcinoma is categorized as low, intermediate and high risk groups which allows us to define the significant cut off points for each group. It can also be helpful in differentiating the groups with different prognosis and management needs.[30,31]

However, there are a few limitations to the current study. Due to small sample size, we were unable to show a significant association. Secondly, more advanced robust techniques like FISH and PCR may show different results in outcomes due to their sensitivity. Third, several potential biases have been identified that could affect our results such as patients age and PSA levels only at the time of diagnosis were taken that prevented us from making any meaningful conclusion for the serum PSA levels and age of onset of prostate adenocarcinoma. Moreover, only needle biopsies and TURP cases were included in the present study. Therefore, patients who've undergone radical prostatectomy and who may have had more advanced stage were not recruited. Furthermore, needle biopsy and TURP specimens limited us for comparative analysis of other parameters like histopathological staging. It is also be likely that HER2/neu treatment strategies will be ineffective in treating prostate cancer. However, future studies with large number of cases may prove beneficial to further explore this association of HER2/neu expression with prostate cancer.

CONCLUSION:

It was concluded from the study that HER2/neu was rarely expressed in prostate adenocarcinoma. Her2 /neu expression is present in acinar variant of this tumor which is a common entity. Therefore, it was evident that HER2/neu is not expressed in the same manner like other cancers such as adenocarcinoma of breast.

Author Contribution:
Santosh Kumar Sidhwani: Primary Researcher
Madeeha Sadiq: Writer
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Sobia Hassan: Writer, Statistical Analysis

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