ORIGINAL ARTICLE

EVALUATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF HER2/neu IN PROSTATE ADENOCARCINOMA

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ABSTRACT

BACKGROUND: Prostate cancer is the second most common cancer in men. Diagnosis of prostate cancer mainly depends upon histological examination. Prostate cancer expresses various immune markers, for example HER2/neu.
The objective of this study was to determine and score the immunohistochemical expression of HER2/neu in prostate adenocarcinoma biopsies. We have also compared the association of HER2/neu expression with biological behavior and risk factors of prostate adenocarcinoma.

METHODS: A cross sectional study was carried out using prostate biopsies, clinically suspected of 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. 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: Only one case showed moderate HER2/neu expression. An insignificant statistical difference was observed between HER2/neu expression and prostate adenocarcinoma. The positive case had age more than 60 years with moderately increase in serum PSA levels and was aggressive in nature at the time of diagnosis.

CONCLUSION: Our results indicated that HER2/neu expression is absent or very rare in prostate adenocarcinoma.

KEYWORDS: Prostate, adenocarcinoma, Gleason's Group, Serum PSA, HER2/neu

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INTRODUCTION

Prostate cancer is the second most common cancer and sixth most common cause of death in men globally.[1] In Pakistan, the prevalence of prostate cancer is 5.3 per 100,000.[2] Unfortunately, a vast number of prostate adenocarcinoma on diagnosis presents as a metastatic disease which contributes to a high mortality rate.[3] Several studies have demonstrated the expression of HER2/neu in prostate adenocarcinoma, hypothesizing that; alteration in cellular proliferation, differentiation, survival, migration and angiogenesis brought about by genetically aberrant HER2/neu receptor could contribute to tumor formation.[4,5] Previously, HER2/neu has proved to be a useful prognostic biomarker in several tumors other than prostate; thereby reducing mortality by providing a viable therapeutic option.[6]

Diagnosis of prostate cancer mainly depends upon histopathologic and immunohistochemical examination.[7] Histologically,adenocarcinoma of prostate is characterized by numerous atypical glands lined by a single layer of cuboidal cells with brisk mitosis.[8] The tumor is graded according to Gleason's criteria from 1 to 10 based on the morphological differentiation; with score 1 being minimal aggressive whereas score 10 represents the most aggressive biological behavior.[9]

The expression of HER2/neu in prostate cancer biopsies could provide an opportunity for novel therapeutic intervention for prostate cancer, as implicated in treatment of various other tumors.[10] Therefore, we evaluated the expression of HER2/neu among prostate adenocarcinoma using immunohistochemistry. We also sought to compare the expression of HER2/neu with biological behavior and age of the patient upon diagnosis.

METHODS

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. The cases with a history of metastatic cancer to prostate, biopsies with benign prostatic hyperplasia or any other benign pathology, biopsies diagnosed with prostatic intraepithelial neoplasia and patients on anti-androgen therapy were excluded from the study. After confirming the diagnosis, 77 cases were randomly selected following informed consent along with pertinent clinical history and PSA levels. The representative Gleason's group was assigned by examining multiple levels of Hematoxyline and Eosin stained sections by the consultant histopathologist. The most representative tumor block bearing abundant tumor volume was selected for immunohistochemistry. The study was conducted following ethical approval from ethics review comity (ERC) of Ziauddin University Karachi.

For immunohistochemistry, method described by Signoretti et al was used. Briefly formalin fixed, paraffin embedded tissue blocks were cut into 3µm sections, dipped into a hot water bath and transferred to glass slide followed by treatment with 0.1mol/L citrate buffer in a microwave for 15 minutes for antigen retrieval. A mouse monoclonal antibody (HER2/neu, cell marque) was prepared at dilution of 1:50ml. The preparation was then applied to the tissue section for 45 minutes followed by buffer rinse, followed by enzyme activity which was developed separately. The 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. The slide was then cover slipped with mounting media. Known HER2/neu positive breast adenocarcinoma slides used as positive controls while tissue slides incubated with tris buffer (TBS) without primary antibodies were served as negative controls.

The immunohistochemistry sections were examined by the same consultant histopathologist to evaluate HER2/neu staining pattern. The tissue sections were considered positive by dark brown cytoplasmic staining in the tumor cells observed by light microscopy. Statistical analysis was performed by SPSS version 21.0 (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 <0.05 was used as cut off limit for statistical significance.

RESULTS

We were able to perform HER2/neu immunohistochemical analysis on all 77 prostate adenocarcinoma biopsies and determined that only one case was HER2/neu positive. This accounts for an insignificant statistical link with prostate adenocarcinoma. Table 1 presents the HER2/neu expression in prostate adenocarcinoma specimens. The strength of protein expression was observed 2 on scale of 3. Figure 1 represents (A) H& E of prostate adenocarcinoma of Slide 9 with variable sized neoplastic glands, (B) moderate immunohistochemical expression of HER2/neu protein in neoplastic glands (C) immunohistochemistry of slide PA 46 with negative expression of HER/neu in neoplastic prostate adenocarcinoma glands and (D) Immunohitochemical expression in control group of Breast adenocarcinoma

Table 1: HER2/neu expression in all study subjects.

HER2/neu	Prostate Adenocarcinoma
Positive	1
Negative	76

The comparative analysis of prostate adenocarcinoma with age showed majority of cases was above 60 years of age upon diagnosis. The mean age of the patients was estimated to be 68.7±7.9 years. Additionally, most tumors presented with high serum PSA levels and aggressive biological behavior. However, no correlation was observed between HER2/neu and these parameters. Table 2 displays the correlation of HER2/neu with different risk factors of prostate adenocarcinoma.

Table 2: Association of HER2/neu with demographic and clinical characteristics in prostate adenocarcinoma.

HER2/neu n=77	Age (years)			PSA level (ng/ml)				Gleason's Group			
	≤ 60	> 60	p-value[1]	< 4	≥ 20	> 50	p-value[3]	Mild Risk[a]	Intermediate Risk[b]	High Risk[c]	p-value[4]
Positive n=1	0	1		1	0	0		0	1	0	
Negative n=76	12	64	0.84*	72	4	0	0.94*	11	20	45	0.25[d]
Total n=77	**12**	**65**		**73**	**4**	**0**		**11**	**21**	**45**	

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

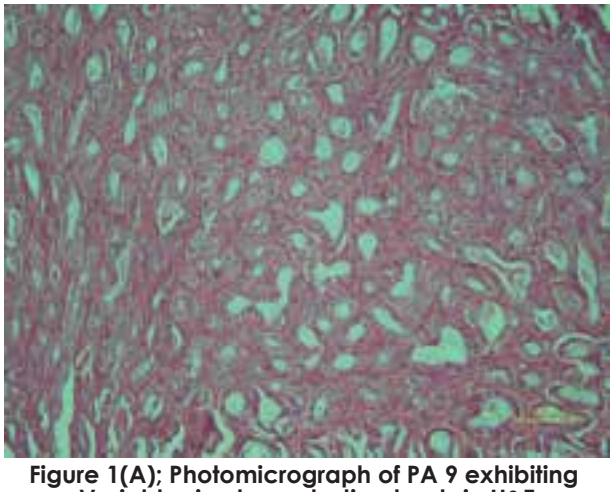

Figure 1(A); Photomicrograph of PA 9 exhibiting Variable sized neoplastic glands in H&E

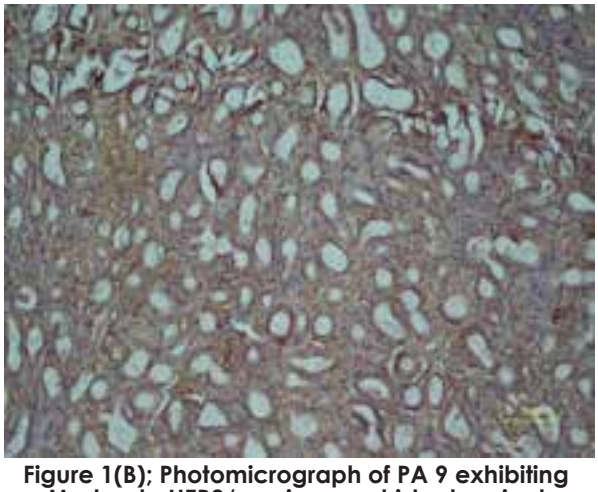

Figure 1(B); Photomicrograph of PA 9 exhibiting Moderate HER2/neu immunohistochemical expression in a typical glands

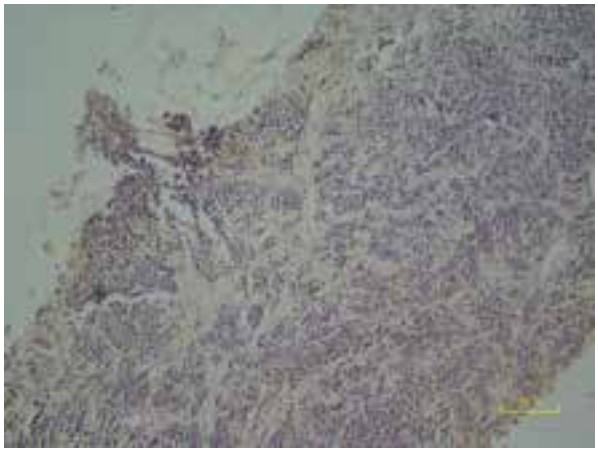

Figure 1(C); Photomicrograph of PA 46 exhibiting HER2/neu negative PA 46 with undifferentiated neoplastic glands

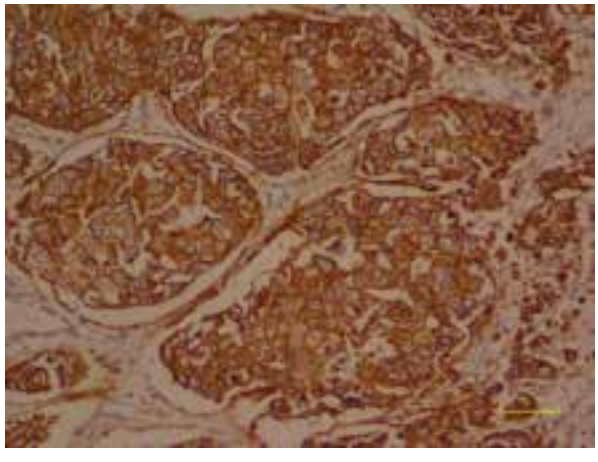

Figure 1(D); HER2/neu membrane immunohistochemical expressions in breast adenocarcinoma control

DISCUSSION

Adenocarcinoma of prostate is the second leading cancer of men globally with high mortality.[11] The incidences of this tumor are on the rise over the last few decades, challenging early diagnosis and therapeutic intervention.[12,13] Previously, some recent studies have documented HER2/neu expression up to 53% of the prostate cancers. Whereas, others fail to document the expression of this biomarker in their series. HER2/neu has an established therapeutic and prognostic significance in other tumors such as adenocarcinoma of breast.[14,18] However, HER2/neu expression was almost nonexistent in our study subjects apart from a single case with moderate level of expression. Our finding is consistent with several reported findings of either no or only very rare expression of HER2/neu in specimen of prostate adenocarcinoma.[19] We suggest that the differences may have been contributed by different antibody or variation in technical skills used in present research.[17] Alternatively, demographic profile, variation in genetic and variation in risk factors of the tumor or tissue fixation and processing may have affected the staining levels.[17, 20]

The strongest risk factor of prostate adenocarcinoma is advanced age. The mean age observed in the present study is 66 years.[21] Numerous studies on large populations with high and moderate burdens of prostate adenocarcinoma indicated that prostate cancer is more consistent in men of 65 years and above (average 70 years) and with a twofold increase of risk after seventies.[22] In present studies, we found the mean age of the study subjects diagnosed with adenocarcinoma of prostate was 68.7±7.9 years.[23] This finding is consistent with the investigation of Burgri et al, who examined different factors in population from south Karachi.[24] However, cases included in present research were hospital based therefore the conclusion on prostate cancer risk and age cannot be drawn for general population.

Several studies have sought parameters to predict the outcome for patients with prostate cancer. Certain histological data – Gleason score; the presence and percentage of a tertiary, less differentiated Gleason pattern, serum PSA levels and tumor stage are considered as the best prognosticators of tumor progression.[25, 26] Additionally, new Gleason subgrouping provides a better opportunity for comparison and assessment of the effect of morphological differentiation, invasion and growth pattern and structure of different prostatic carcinoma.[27] Furthermore, the subdivision into low, intermediate and high risk groups; as recommended by new guidelines can allow meaningful cut off points to be defined and can be used to help differentiate patient's groups with different prognosis and therefore different management needs.

Association between HER2/neu overexpression and aggressive nature of tumor proliferation have been described for instances in breast cancer and endometrial carcinoma.[22] In the present study, a HER2/neu positive case bears no statistical correlation with histological parameter (Gleason group) or elevated serum PSA level. This finding may have been accounted by an extremely low prevalence of HER2/neu positive cases in our series. We believe that a study with more robust technique such as HER2/neu gene amplification, using a larger series of prostate adenocarcinoma may result in a different outcome. It is therefore not surprising that no association between HER2/neu expression and clinicopathological parameters could be observed in this study.

There are several limitations in the present study. Firstly, limited number of cases may have prevented us from documenting a significant link. Secondly, a more robust technique such as PCR and FISH could have proven more sensitive in determining our outcomes. Thirdly, there are several sources of potential bias that could have affected our results. In particular, patient's age and serum PSA level at time of diagnosis were taken into account which prevented us to draw a meaningful conclusion regarding the age of onset and serum PSA levels. Furthermore, the cases we analyzed were selected from those who underwent TURP and needle biopsy specimens. Therefore, patients who had undergone radical prostatectomy and presented with more advanced prostate adenocarcinoma were not included. Additionally, the use of TURP and needle biopsy specimens limited us in considering other parameters such as histopathological staging for comparative analysis.

CONCLUSION

Our results indicated that HER2/neu expression is absent or very rare in prostate adenocarcinoma and its variants. Therefore, the expression of this biomarker is not the same as in other cancers such as breast adenocarcinoma. It is also unlikely that HER2/neu treatment strategies will be effective in treating tumor of prostate, although future researches studying a larger number of cases is needed to confirm our outcomes.

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