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HER2/neu Immunohistochemical Overexpression in Prostatic Adenocarcinoma in Relation to Tumor Pathological Behavior

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Abstract: Prostatic adenocarcinoma represents the second most common malignant tumor found in men aged over 65y. The survival rate of cancer prostate (Cap) patients is poor because of the recurrence of the disease. The lack of effective therapies for advanced CaP is largely related to poor understanding of the molecular mechanisms underlying the progression of this disease (invasion and metastasis). Preclinical studies suggest that HER2 expression plays a role in prostate cancer progression. Randomly collected 60 archival formalin fixed paraffin-embedded cases (30 TUR-P biopsies, 26 radical prostatectomy specimens and 4 open prostatectomy specimens) were studied. Immunohistochemical staining was performed using monoclonal mouse anti-human HER2/neu antibody. HER2/neu staining was scored from 0 to 3+; patients with IHC scores of 2+ or 3+ were considered to have positive HER2 overexpression. Results: Nineteen of the sixty cases presented by 31.7% had positive HER2 overexpression (score 2+ and 3+). There was a significant correlation between HER2/neu expression and percentage of the represented tumor tissue as well as tumor stage. Whereas, no correlation was found between HER2/neu immunostaining and age, serum PSA level, Gleason score, capsular invasion or perineural invasion. Conclusion: HER-2/neu overexpression in prostate carcinoma might have a role in tumor invasion, aggressiveness as well as metastatic potentiality. This might shed the light on the role of trastuzumab in the treatment of patients with prostatic adenocarcinoma.

Key words: Prostatic Adenocarcinoma · Gleason score · HER2 · Immunohistochemistry

INTRODUCTION

The staging of prostate cancer (as well as the grade) is highly significant in determining the best options for treatment [1]. In the last years, hormonal therapy has been the mainstay of treatment for advanced prostate cancer. Treatment of metastatic cancer with hormone therapy temporarily controls symptoms in 70–80% patients [2]. Despite recent improvements in diagnosis and therapeutic techniques, the survival rate of cancer prostate (CaP) patients is poor because of the recurrence of the disease [3]. The lack of effective therapies for advanced CaP is related to a large extent to poor understanding of the molecular mechanisms underlying the progression of this disease (invasion and metastasis) [4, 5]. HER2/neu plays a role in oncogenesis through its action on cellular cascades that involve proliferation and differentiation of epithelial cells. Inappropriate signaling may lead to increased/uncontrolled cell proliferation, decreased apoptosis, enhanced cancer cell motility and angiogenesis [6-9]. *In vitro* studies for HER2/neu expression and amplification in primary prostate cancers have been reported. Rates of positivity range from 0 to 100% in immunohistochemical studies and from 0 to 53% in amplification analyses [10].

The aim of the work was to assess HER2 overexpression in cases with prostatic adenocarcinoma and to correlate its expression with tumor behavior including grading and staging.

MATERIALS AND METHODS

Sixty archival formalin fixed paraffin- embedded, routinely processed cases (30 cases as TUR-P biopsies, 26 cases as radical prostatectomy specimens and 4 cases as open prostatectomy specimens) were retrospectively and randomly collected from the Pathology Department, Kasr Al Aini Hospital, Cairo University. For each

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collected case, available clinical data including; age, PSA level and tumor node metastasis (TNM) staging in cases of radical prostatectomy were retrieved from the computer files. Two serial sections from each tissue block were cut at 5 microns thickness. One section was stained by (H&E), for histopathological evaluation and the other one was mounted on positively charged slides for immunostaining using monoclonal mouse anti-human HER-2/neu antibody. Sections were re-evaluated by two independent pathologists.Tumor grade was determined according to modified *Gleason grading system, 2005* [11].

Interpretation of Staining: Only the membrane staining intensity and pattern was evaluated as following: complete cytoplasmic membrane staining of weak or moderate intensity in at least 10% of cells is considered 2+ (weak positive), whereas a strong membrane reaction in more than 30% of cells is recorded as 3+ (strong positive). Incomplete membrane staining (1+) as well as no staining are considered negative [12]. Reaction intensity was compared with that in the control cell lines provided in the kit (HER2 positive breast carcinoma).

Statistical Analysis: Fisher exact and chi-square tests were used in the analysis. The significance of the results was assessed by determining the probability factor "P" value. P value of < 0.05 was considered significant.

RESULTS

In the present study, the mean age of prostatic adenocarcinoma patients was 67.38 (ranging between 38 - 88 years), with a count of 42 cases (70%) above the age of 60. Gleason grade 4 was the most common grade among the collected cases (32 cases representing 53.3%) while, grade 2 was the least common (only 3cases representing 5%). The mean Gleason score (GS) was 7.83 with a higher frequency for GS 9(21 cases; 35%) distributed as: 15 cases with Gleason grade (4+5) and 6 cases with Gleason grade (5+4). Gleason score 7 was the second most common (18) cases; 11 cases had Gleason grade (3+4) and 7 cases had Gleason grade (4+3). GS 5 as well as GS 10 showed the lowest frequency; only three cases for each. For statistical analysis, the tumors were considered well differentiated when GS was ≤ 6, moderately differentiated when the GS was 7 and poorly differentiated when the GS was 8 to 10. Over all, perineural invasion (PNI) was detected in 22 cases (18 out of which were radical prostatectomies) and absent in 38 cases (63.3%). 11 cases (18.3%) out of 60 cases [distributed as 9 radical and 2 open prostatectomy specimens] showed positive capsular invasion by tumor tissue. In the 26 radical prostatectomy cases, the disease was confined to the gland in 18 patients (69.3%) staged as pT2 (a, b, c). The tumor extended beyond the prostate; stage pT3 (a, b) in the remaining 8 patients (30.7%) (Fig. 1 A). Lymph nodes were excised bilaterally in 23 cases with positive metastases in 4 cases having lymph node stage N1; 3 cases of which showed tumor stage T3b and stage T2c in one case (Fig.1B). We found significant statistical relationship between the age and Gleason score *(P-value= 0.043)*, perineural invasion and Gleason score in radical prostatectomy cases *(P value =0.006)* as well as perineural invasion and the pT stage *(P value<0.005)*.

Regarding HER2/neu overexpression, 37 specimens (61.7%) had detectable membranous staining. 19 tumors (31.7%) had positive HER2/neu overexpression (score 2+ and 3+); these represented as (10) TURP and (9) radical prostatectomy specimens (Table 1). In this work,

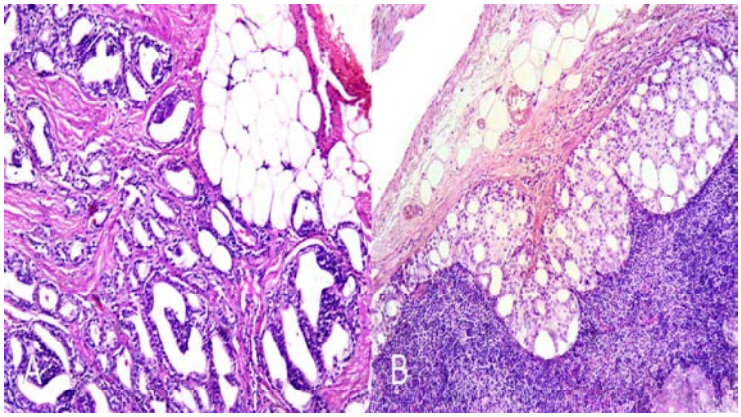


Fig. 1(A): Malignant prostatic acini are seen reaching the extra prostatic adipose tissue pT3a (H&E X100), (B) section in lymph node showing subcapsular malignant tissue deposit formed of neoplastic acini showing cribriform pattern (H&E X100)

Table 1: Distribution of HER2/neu overexpression in the collected cases

			HER2/neu score				
			0	1+	2+	3+	Total
Specimen	open prostatectomy	Count	3	1	0	0	4
		% of Total	5.0%	1.7%	0.0%	0.0%	6.7%
	radical prostatectomy	Count	12	5	3	6	26
		% of Total	20.0%	8.3%	5.0%	10.0%	43.3%
	TUR-P	Count	8	12	7	3	30
		% of Total	13.3%	20.0%	11.7%	5.0%	50.0%
Total		Count	23	18	10	9	60
		% of Total	38.3%	30.0%	16.7%	15.0%	100.0%

Table 2: Relation between HER2/neu overexpression and TNM stage in the radical cases

			HER2/neu score				
Pathologic stage			0	1+	2+	3+	Total
Stage T2	T2aNx	Count	0	0	2	1	3
		% of Total	0.0%	0.0%	7.69%	3.85%	11.54%
	T2aN0	Count	2	0	0	2	4
		% of Total	7.69%	0.0%	0.0%	7.69%	15.38%
	T2bN0	Count	0	0	0	2	2
		% of Total	0.0%	0.0%	0.0%	7.69%	7.69%
	T2cN0	Count	5	2	1	0	8
		% of Total	19.23%	7.69%	3.85%	0.0%	30.77%
	T2cN1	Count	0	0	0	1	1
		% of Total	0.0%	0.0%	0.0%	3.85%	3.85%
Stage T3	T3aN0	Count	0	2	0	0	2
		% of Total	0.0%	7.69%	0.0%	0.0%	7.69%
	T3bN0	Count	2	1	0	0	3
		% of Total	7.69%	3.85%	0.0%	0.0%	11.54%
	T3bN1	Count	3	0	0	0	3
		% of Total	11.54%	0.0%	0.0%	0.0%	11.54%
Total		Count	12	5	3	6	26
		% of Total	46.15%	19.23%	11.54%	23.08%	100.0%
P value	0.011						

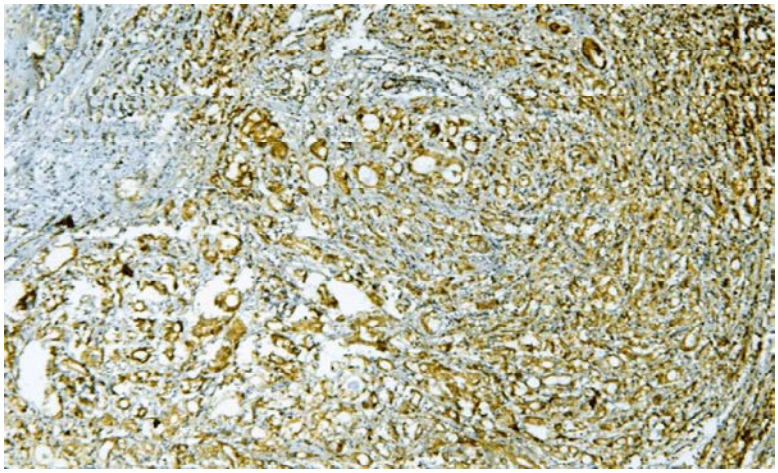
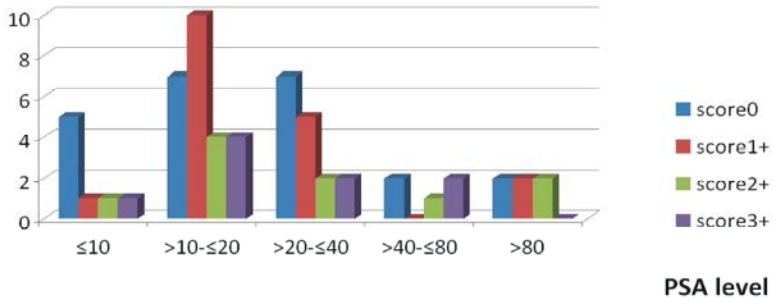
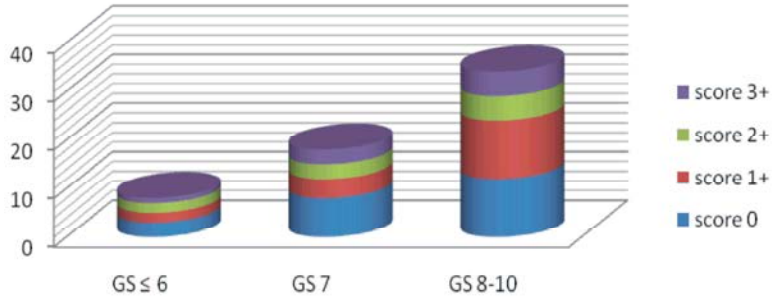


Fig. 2: Prostatic adenocarcinoma: malignant prostatic acini involving 90% of the represented tissue score 3+HER2/neu immunostaining (X100).

we found a statistically significant correlation between HER2/neu immunostaining and extent of tumor *(P value 0.013)* as well as tumor stage *(P value 0.011)* (Table 2) (Fig. 2). Although, PSA level in 25 cases (41.7%) was above 10 up to 20ng/ml; we did not find significant statistical correlation between its level and HER2/neu staining intensity, similarly; no significant correlation could be detected regarding the Gleason's score of our



Graph 1: Relation between serum PSA value and HER2/neu overexpression



Graph 2: Correlation between HER2/neu overexpression and the Gleason score

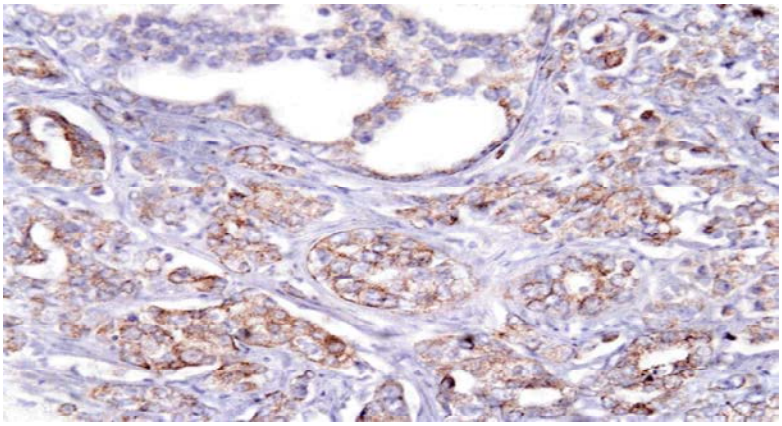


Fig. 3: Prostatic adenocarcinoma; cribriform growth pattern showing score1+ HER2/neu immunostaining (X200).

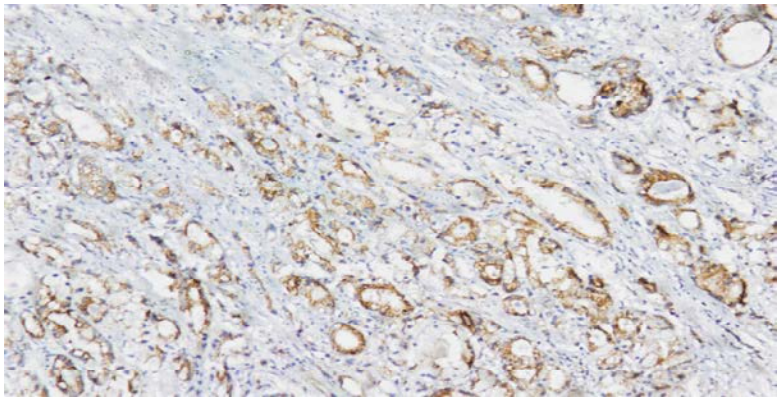


Fig. 4: Prostatic adenocarcinoma; Gleason grade 3 showing score 2+ HER2/neu immunostaining (X100).

cohort (Graphs 1, 2) (Figs. 3 & 4). Additionally, there was no significant statistical association between HER2/neu overexpression and perineural or capsular invasion *(P value= 0.647, 0.920, 0.428 and 0.275, respectively)*.

DISCUSSION

Androgen deprivation therapy (ADT) was initially very effective in patients with cancer prostate, eventually it was noted that patients might progress into castrate resistant prostate cancer (CRPC). The failure of ADT was notable with disease progression associated with rising PSA levels, clinical symptoms or abnormal scans [13]. In our cohort more than half of the studied cases (56.7%) obtained GS of 8 to 10 (high-grade tumors), followed by 18 (30%) cases with moderately differentiated tumors (GS 7). These results are much closer to different studies that found that the majority of cases (60 and 60.6%) were presented with high Gleason scores of 7 to 10 [14, 15]. Meanwhile, *Hussain et al.* [16] and Forae and Aligbe [17] reported that moderately differentiated prostatic carcinoma with (Gleason's score 5-7) was the most common accounting for 56 and 59.8%, respectively. Moreover, only 19 of our studied cases (31.7%) had positive HER2/neu overexpression (scores 2+ and 3+). Different studies showed wide variation with HER2/neu overexpression; ninety-nine (66%) out of 150 radical prostatectomy cases were positive for HER2/neu, others revealed that 33 out of 216 and 20 of 150 cases of radical prostatectomy specimens presented by 15 and 13.3% respectively were positive for HER2/neu [18-20]. Studies on cases with progressive CRPC revealed that 4 out of 39 cases presented by 10% of radical prostatectomy specimens had positive HER2/neu immunostaining (2+ to 3+ staining) [21]. Similarly, results were notable on cases with metastatic prostate carcinomaas positive HER2/neu overexpression was seen in only 10 out of 104 cases (9.6%) [22]. Despite the positive correlation between the measured prostate volume and the PSA values proved [23], we could not find significant correlation between HER2/neu expression and the preoperative serum PSA level. That might be due to increased serum PSA level in many cases like prostatitis, hyperplasia, tumors, after catheterization and after ejaculation [10, 22-24]. The correlation between Gleason score and HER2/ neu was controversial as in our study and different studies failed to record significant correlation [18, 20, 24, 25], although, there was strong association between HER2/neu immunostaining and high-grade tumors (Gleason score 8-10) [10, 19, 22]. In other instance, the correlation between HER2/neu overexpression and the pathological stage in our studied cases was statistically significant. This result disagrees with Fonseca *et al.* [18] and Calvo *et al*. [24] who noted no association between HER2/neu expression and tumor stage. Nevertheless, it much agrees with previous studies recorded that HER2/neu immunoreactivity in 28 out of 42 cases staged as pT2 and in 9 out of 16 cases staged as pT4N+ with statistically significant correlation between HER2/neu immunostaining and pT stage [10, 26]. The reported variations between different studies on HER2/neu expression are probably due to different patient inclusion, receptor-scoring criteria and the differences in immunohistochemical retrieval techniques between the laboratories. HER2/neu is thought to have an active participation in the first steps of local carcinogenesis. Furthermore, the possibility that etiological differences play a role in the variation of reported HER2/neu expression levels cannot be excluded.

CONCLUSIONS

HER2/neu overexpression in cancer prostate might lead to unfavorable prognostic outcome as it might have a role in tumor aggressiveness, especially with tumor pathologic stage. Although we could not find any significant correlation with PSA level, tumor grade, capsular invasion or perineural invasion, this study might shed the light on the role of HER2/neu. These findings might be of importance considering the use of trastuzumab as a single agent or in combination with chemotherapy in the treatment of patients with prostatic adenocarcinoma, especially those with metastasis. Finally, we recommend trials to find the role of HER2/neu in castrate resistant prostate cancer (CRPC) patients.

REFERENCES

1. American Cancer Society, 2012. Prostate cancer: Early Detection, Diagnosis and Staging TOPICS. Cancer.org, 3: 25-85.
2. Carles, J., J. Lloreta, M. Salido, A. Font, M. Suarez, V. Baena, M. Nogue, M. Domenech and X. Fabregat, 2004. Her-2/neu Expression in Prostate Cancer A Dynamic Process? Clin. Cancer Res., 10: 4742- 4745.
3. Klein, E.A. and I.M. Thompson, 2004. Update on chemoprevention of cancer prostate. Curr. Opin. Urol., 14: 143-149.
4. Saleem, M., V. M. Adhami, W. Zhong, B.J. Longley, C.Y. Lin, R.B. Dickson, S. Reagan-Shaw,D.F. Jarrard and H. Mukhtar, 2006. A novel biomarker for staging human prostate adenocarcinoma: overexpression of matriptase with concomitant loss of its inhibitor, hepatocyte growth factor activator inhibitor-1. Cancer Epidemiol Biomarkers Prev., 15: 217-227.
5. Anis, I., H. N. Hosni, M.F.Darweesh and M. Abd El Rahman, 2013. Immunohistochemical Expression of Cyclin D1 in Egyptian Patients with Prostatic Carcinoma.World J. Med. Sci., 8(4): 306-313.

6. Bargmann, C.I., M.C. Hung and R.A. Weinberg, 2012. The neu oncogene encodes an epidermal growth factor receptor-related protein. Nature (Lond.), 319: 226-230.
7. Linggi, B. and G. Carpenter, 2012. ErbB receptors: new insights on mechanisms and biology. Trends Cell Biol., 16: 649-656.
8. Siampanopoulou, M., N. Diamsis, G. Galaktidou and G. Psarrakou, 2013. Profiling serum HER-2/neu in prostate cancer. Hippokratia, 17(2): 108-112.
9. Abdelhamid, M., S. Abdelaziz, S. Daoud, A. Sadat, A. Abdelhaseeb, T. Nabil, M. Abdelbasset, H. Nafady, K. A. Shawky and M. Abdelmoola, 2014. Concordance of HER-2/Neu over Expression with Steroid Receptor Status in Female Breast Cancers. Surgical Science, 5: 354-362.
10. Minner, S., B. Jessen, L. Stiedenroth, E. Burandt, J. Köllermann, M. Mirlacher, A. Erbersdobler, C. Eichelberg, M. Fisch, T.H. Brümmendorf, C. Bokemeyer, R. Simon, T. Steuber, M. Graefen, H. Huland, G. Sauter and T. Schlomm, 2010. Low Level Her2 Overexpression Is Associated with Rapid Tumor Cell Proliferation and Poor Prognosis in Prostate Cancer. Clin Cancer Res., 16(5): 1553-1560.
11. Epstein, J.I., W.C. Allsbrook, M.B. Amin and L.L. Egevad, 2005. ISUP Grading Committee. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am. J. Surg. Pathol., 29: 1228-1242.
12. Nordic immunohistochemical Quality Control, 2013. HER-2. Nordi QC.
13. Vaishampayan, U., A. Thakur, R. Rathore, N. Kouttab and L. Lum, 2015. Phase I Study of Anti-CD3 x Anti-Her2 Bispecific Antibody in Metastatic Castrate Resistant Prostate Cancer Patients. Prostate Cancer, pp: 285193.
14. Catalona, W.J., J.A. Anterior, K.A. Roehi and J.W. Moul, 2002. Screening for prostate cancer in high-risk population. J. Urol., 168: 1980-1983.
15. Obiorah, C.C. and S.O. Nwosu, 2011. A histopathological study of carcinoma of the prostate in Port Harcourt, Nigeria, 14(3): 363-367.
16. Hussain, I., A.M. Khattak, S.H. Shah, K. Salim and J. Qamimer, 2005. Prostatic cancer: A retrospective study of 50 patients. Biomedica, 21: 44-47.
17. Forae, G.D. and J.U. Aligbe, 2014. Histopathological patterns of prostate cancer in an African population: A private practice experience. Trop. J. Med Res., 17(1): 16-19.
18. Fonseca, G.N., M. Srougi, K.R. Leite, L.J. Nesrallah and V. Ortiz, 2004. The role of HER2/neu, BCL2, pp: 53 genes and proliferating cell nuclear protein as molecular prognostic parameters in localized prostate carcinoma. Sao Paulo Med J., 122(3): 124-127.
19. Jorda, M., A. Morales, Z. Ghorab, G. Fernandez, M. Nadji and N. Block, 2002. HER2 expression in prostatic cancer: A comparison with mammary carcinoma. J Urol., 168: 1412-1414.
20. Mofid, B., M.J. Nodushan, A. Rakhsha, L. Zeinali and H. Mirzaei, 2008. Relation between HER-2 Gene Expression and Gleason Score in Patients with Prostate Cancer. Urol J., 4: 101-104.
21. Reese, D.M., E.J. Small, G. Magrane, F.M. Waldman, K. Chew and D. Sudilovsky, 2001. HER2 Protein Expression and Gene Amplification in Androgen-Independent Prostate Cancer. Am. J. Clin Pathol., 116: 234-239.
22. Dai1, B., Y. Kong, D. Ye1, C.G. Ma, X.Y. Zhou and X.D. Yao, 2008. Human epidermal growth factor receptor type 2-protein expression in Chinese metastatic prostate cancer patients correlates with cancer specific survival and increases after exposure to hormonal therapy. Asian J Androl, 10(5): 701-709.
23. Karera, A., E.F. Maguranyanga, S. Nleya and G. Chingarande, 2014: The Effectiveness of Ultrasound in Early Detection of Benign Prostatic Hypertrophy; A Case Study of Chitungwiza, Zimbabwe.World J. Med. Sci., 10(2): 122-128.
24. Calvo, B.F., A.M. Levine, M. Marcos, Q.F. Collins, M.V. Iacocca, L.S. Caskey, C.W. Gregory, Y. Lin, Y.E. Whang, H.S. Earp and J.L. Mohler, 2003. Human Epidermal Receptor-2 Expression in Prostate Cancer. Clin Cancer Res., 9: 1087-1097.
25. Rodrigues, S.R., M. Yabiko, R.B. Thomaz, T. Theodoro, B. Alves, L.A. Azzalis, E.C. Pereira, V.B. Junqueira, R. Corazzini, F. Adami and F.L. Fonseca, 2013. E-Cadherin, beta-catenin and HER2 expression in prostate cancer tissues with perineural invasion and their correlation with Gleason score: A preliminary study. Academic Journal, 8(6): 194-198.
26. Di Lorenzo, G., G. Tortora, F. B.D'Armiento, G. De Rosa, S. Staibano, R. Autorino, M. D'Armiento, M. De Laurentiis, S. De Placido, G. Catalano, A.R. Bianco and F. Ciardiello, 2002. Expression of Epidermal Growth Factor Receptor Correlates with Disease Relapse and Progression to Androgen-independence in Human Prostate Cancer. Clin. Cancer Res., 8: 34-38.