Pattern of HER2 Overexpression in Urinary Bladder Carcinomas in Kano, Nigeria

Muhammad Sanusi Haruna[1], *Akinfenwa Taoheed Atanda*[2], *Yawale Iliyasu*[3]

1Department of Morbid Anatomy and Forensic Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria

2Department of Pathology, Bayero University, Kano, Nigeria

3Department of Pathology, Ahmadu Bello University, Zaria, Nigeria

Correspondence to: Muhammad Sanusi Haruna; email:haruna.muhammad@udusok.edu.ng.

Received: 13 Apr 2021; Revised: 14 Dec 2022; Accepted: 17 Dec 2022; Available online: 21 Dec 2022

Summary

Background: The worldwide distribution of bladder cancer varies, conforming to differences in environmental and genetic risk factors. HER2 neu is overexpressed in many human cancers, includingurinary bladder carcinomas. The aim of this study is therefore to evaluate the pattern of HER2 positivity, and correlate HER2 positivity of urinary bladder carcinomaswith age, gender of patients, histological subtypes, and tumor grades. **Methods:** This is a 2-year retrospective study from January 2015 to December 2016. Patients' clinicopathological information was extracted from their case folders and pathology reports. The histological subtyping using the WHO 2016 classification and grading was done and then reviewed by authors. HER2 scoring was done using the recommendations of the American Society of Clinical Oncology/College of American Pathologists. **Results:** Sixty cases of bladder cancer were diagnosed during the study period. HER2/neu positivity (3+) was observed in 24 (40%) of all the cases. Statistically significant association was observed between HER2 neu protein overexpression and increasing tumor grade ($p \leq 0.001$). **Conclusion:** This study recorded HER2 overexpression in 40% of study subjects. There is a statistically significant association between HER2 overexpression and increasing tumor grade.

Keywords: Bladder, HER2, Overexpression, Carcinoma

Ann Afr Surg. 2023; 20(1): 7-13

DOI: http://dx.doi.org/10.4314/aas.v20i1.3

Conflict of interest: None

Funding: None

Introduction

Worldwide, cancer of the urinary bladder is a heterogeneous disease with wide geographical variation in age, gender, prevalence, and histological subtypes (1). It is the second most common urologic malignancy, 9th in global cancer incidence, and the 17th most common cancer with a mortality incidence of 3.2/10,000 and 0.9/100,000 in males and females, respectively (1). A combination of negative factors synergize to bring about a dismal outcome for individuals diagnosed with bladder cancer. These include late presentation, recurrence,

bulky tumors, and metastasis at the time of presentation (2,3). As a matter of fact, the poor responses and decrease in survival rates recorded with conventional chemotherapy dictate the need for the search of specific molecular agents that would target tumor cells (4). One of these targets is a member of the epidermal receptor family, HER2/neu.

HER2/neu, a proto-oncogene, is a transmembrane 185 kDa protein with its encoding gene located on chromosome 17q21 (5). The HER2/neu proto-oncogene is a member of the epidermal receptor family and is normally involved in the regulation of cell proliferation, survival, and motility (6). It is overexpressed in many human cancers (6). Overexpression of HER2 protein/HER2 gene amplification has been recently associated with poor outcomes in these malignancies (7).Furthermore, HER2 gene application and protein overexpression are regarded as prognostic markers for patients with recurrent progressive bladder tumors (7).Therapeutic strategies targeting EGFR (and HER2) are being developed, and the pattern of HER2 overexpression is not known in the majority of the African population, including that of Kano, Nigeria, and thus the need to evaluate the pattern in this setting. The aim of this study is therefore to evaluate the pattern of HER2 positivity in carcinoma of the urinary bladder in the population of Kano, in north-western Nigeria, to evaluate the proportion of urinary bladder carcinomas with HER2 positivity, and to correlate HER2-positive urinary bladder carcinomas with different histological subtypes such as tumor grade, age, gender, and comorbidity with schistosomal infection.

Materials and Methods

This is a retrospective cross-sectional study undertaken from January 2015 to December 2016, and includes all carcinomas of the urinary bladder diagnosed in the pathology department of Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria. The materials included for this study consist of all histologically confirmed cases of urinary bladder carcinomas diagnosed in the histopathology laboratory of AKTH, Kano,Nigeria,within the study period. All nonepithelial bladder cancers and all cystoscopic biopsy cases in which tissues are exhausted were excluded from this study.

Patients' clinicopathological information was extracted from their case folders and pathology reports and this included age, sex, tumor size, tumor growth pattern, and presence of carcinoma *in situ* and ova of Schistosoma or the adult organism. Histological subtyping using the World Health Organization (WHO) 2016 classification and grading was done and subsequently reviewed by the authors.

Finally, tissue sections for immunohistochemistry were cut at 3 µm, floated onto APES (aminopropyltriethoxysilane)-coated slides, blotted, heat-fixed onto the slides at 70°, dewaxed, and rinsed in running tap water and distilled water. Antigen retrieval was done using citrate buffer in a pressure cooker for 25 min. Endogenous peroxidase activity was blocked, rinsed in phosphate buffered saline (PBS), and then treated with UltraV protein block for 5 min to block for nonspecific background staining (NSB). The NSB was then drained from the slides, diluted primary antibody added for 45 min, rinsed thoroughly with (PBS/Tween), and primary antibody enhancer added for 10min. Horseradish peroxidase (HRP) polymer was applied for 10 min followed by rinsing in PBS/Tween and distilled water; application of chromogenic substrate, and immersion in 1% copper sulfate was followed for 5min each. The slides were then washed under running tap water, counterstained in hematoxylin, dehydrated, cleared, and mounted.

HER2 scoring was done using recommendations of the American Society of Clinical Oncology/College of American Pathologists. Cases with a score of 2+ were considered as negative.

All lesions with dyscohesive cells with architectural disarray exhibiting large hyperchromatic nuclei and frequent abnormal mitotic figures are to be considered as high grade. By contrast, lesions with orderly arranged, evenly spaced cells with mild variation of nuclear size and shape, and infrequent mitotic figures are to be considered as low-grade lesions.

Statistical analysis was performed using thestatistical package for social sciences (SPSS) version 13. Chi-square and Fisher exact test were used for evaluation of

the statistical significance of associations between HER2 overexpression and clinicopathological parameters. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated where appropriate.
Statistical significance was considered at a *p*-value of <0.05. Tables and figures were used for further characterization of the results.

Ethical consideration

A proposal was first sent to the Ethical Committees of AKTH, Kano, Nigeria, and approval for the study was obtained (Ethical approval No: AKTH/MAC/SUB/12A/P-3/VI/2062.

.

Results

Of the 60 carcinomas of the bladder seen in the study period, 36 (60%) cases were transitional cell carcinomas (TCC), which constituted the bulk of the malignancies. These were followed in frequency by 21 (35%) cases of squamous cell carcinoma (SCC).

Table 1. Gender and age distribution of the urinary bladder carcinomas relative to the histological subtypes

HISTOLOGICAL SUBTYPES	AGE IN YEARS						TOTAL(%)
	24–35	36–45	46–55	56–65	66–75	76–85	
AC							
M				3			3(5%)
F							
SCC							
M	3	4	4	2	3		16(26.7%)
F		1		2	1	1	5(8.3%)
TCC							
M	3	3	5	9	6	5	31(51.7%)
F		1	1	1		2	5(8.3%)
Total	6	9	10	17	10	8	60(100%)

.

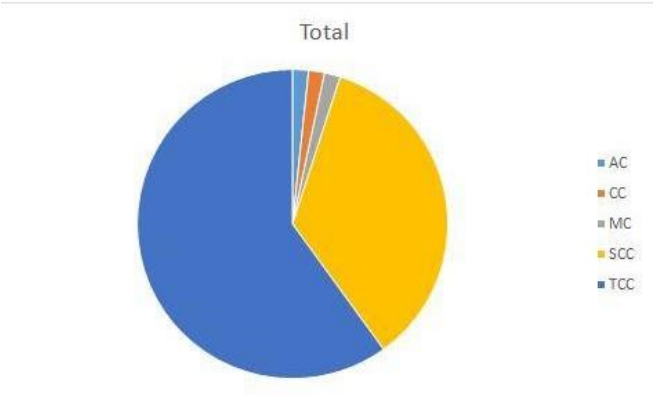

Figure 1. Figure 1. Pie chart showing Histological subtypes of Urinary bladder carcinoma in Kano

AC= Adenocarcinoma, CC clear cell adenocarcinoma, MC mucinous Adenocarcinoma, SCC squamous cell carcinoma, TCC transitional cell carcinoma

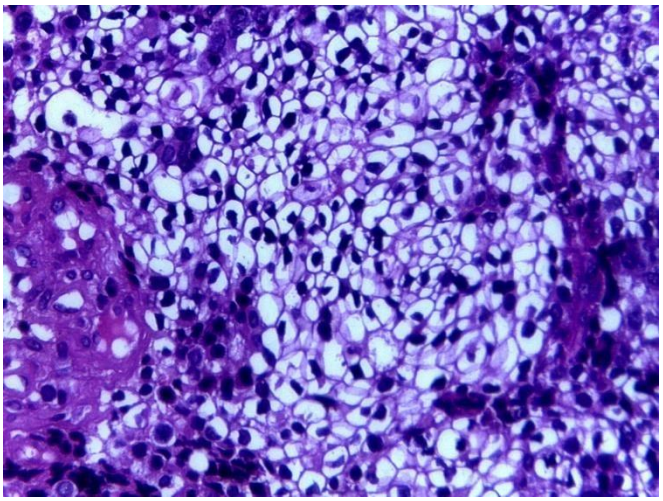

Figure 2**.** Clear cell Adenocarcinoma of urinary bladder (H and E, x 10)

Adenocarcinomas (ACs) were the least frequent and accounted for only 3 (5%) cases (Table 1 and Figure 1-2).

HER2/neu positivity (3+) was observed in 24 (40%) of all the cases, while the remaining 36 (60%) were HER2-negative (2+, 1+, and 0). HER2 positivity was also seen more frequently in the age groups of 45–54 and 55–64 years, each with a frequency of six cases and representing 10% each of the positive cases. The least positivity was recorded in the 25–34year age group with only one positive case (1.6%).

Table 2. Pattern of HER2 staining of carcinomas of the bladder

HER2 SCORES	AC	SCC	TCC	TOTAL(%)
Positive(3+)	0	9	15	24(40%)
Negative(0,1+,2+)	3	12	21	36(60%)
Total	3	21	36	60(100%)

A total of 20cases (83.3%) with HER2 positivity were among males while 4 (16.7%) were among females;15(41.7%) of the 36 cases of TCC were HER2-positive while the remaining 21(58.3%) were negative. Among the 21 cases of SCC, 9 (42.9%) were positive while the remaining 12 (57.1%) were negative. All three ACs were HER2-negative (Table 2 and Figures3–6).

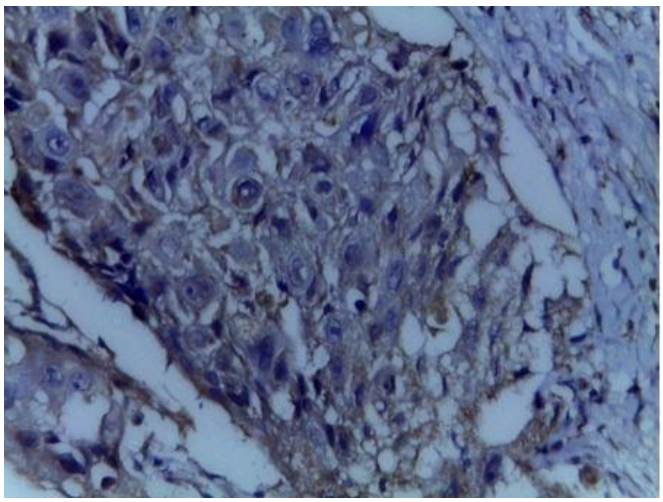

Figure 3. HER2 Neu faint membrane Staining 1+ (HER2 Immunohistochemistry x 100)

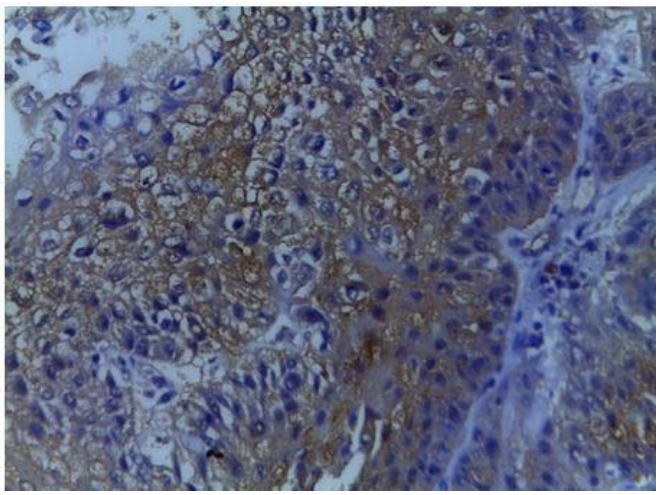

Figure 4. HER2 Neu membrane Staining 2+ (HER2 Immunohistochemistry x 100)

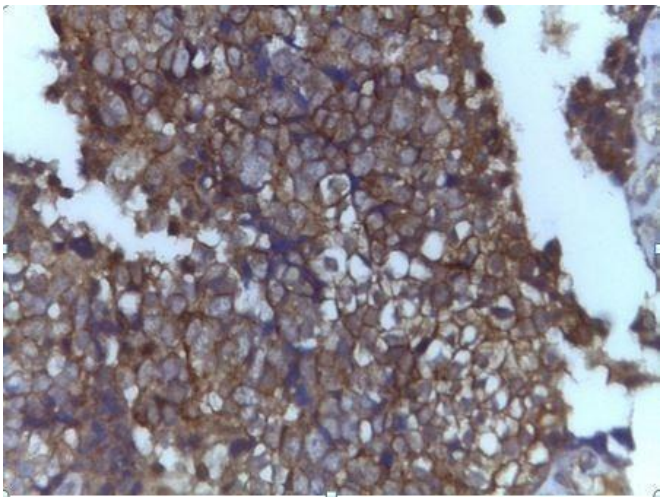

Figure 5. HER2 Neu complete membrane staining. 3+ (HER2 Immunohistochemistry x 100)

Positivity for HER2 staining was noted in 3 (27.3%) of the 11 cases with schistosomal ova. The remaining 8 (72.7%) cases with schistosomal ova were HER2-negative.

Overexpression of HER2 was also noticed in 20 (62.5%) of the 32 high-grade lesions and 9 (36.0%) of the 25 low-grade carcinomas. It was, however, negative in 7 (21.9%) and 16 (64.0%) cases of high- and low-grade tumors, respectively.

Statistically, a significant association was observed between HER2 neu protein overexpression and tumor grade ($p \leq 0.001$), high grade compared to low grade: OR10.02; 95% CI: 2.79–35.89.

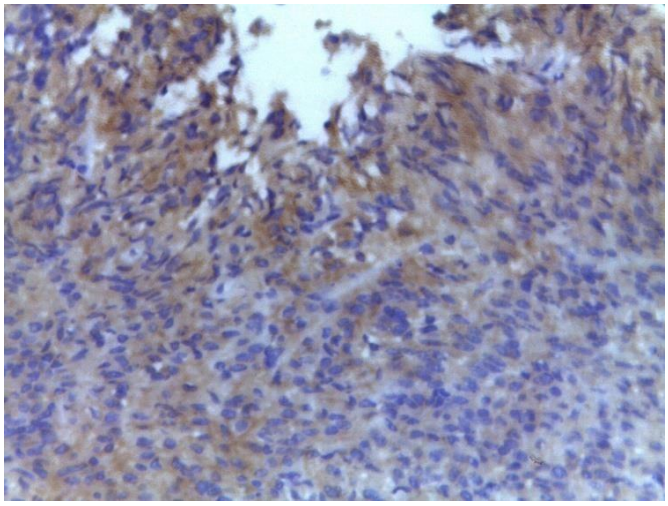

Figure 6. HER2 Neu background staining. 0 (HER2 Immunohistochemistry x 100)

However, no significant association was noted between HER2 neu protein overexpression and tumor histological subtype, presence of schistosomal ova, age, and gender (Table 3).

The age and gender distribution of cases are shown in Table 2. Photomicrographs of HER2 neu immunohistochemical staining are shown in Figures 3–6.

Table 3. HER2 neu overexpression in relation to patient's age, gender, histological subtypes tumor grade, and presence of schistosomal ova

HER2 NEU OVEREXPRESSION			
	POSITIVE	NEGATIVE	p-VALUE
Age(years)			
<55	12(20.0%)	13(21.7%)	0.211
>55	12(20.3%)	23(38.3%)	
Gender			
Male(50)	20(33.3%)	30(50%)	0.642
Female(10)	4(6.7%)	6(10%)	
Histology			
TCC	15(25.0%)	21(35.0%)	0.208
SCC	9(15.0%)	12(20.0%)	
AC	0	3(5%)	
Grade			
High	20(33.3%)	12(20.0%)	0.0001
Low	4(6.6%)	24(40.0%)	
Carcinoma with schistosomal ova			
Present(12)	9(15%)	3(5%)	0.163
Absent(48)	26(43.3%)	22(36.6%)	

Discussion

Immunohistochemical study results revealed HER2 neu protein overexpression in 24 (40.0%) patients (Tables 3 and 4). Overexpression of HER2 Neu has been reported in several human cancers with poor clinical outcomes, including cancer of the urinary bladder. There are variations in the incidence of HER2/neu positivity in urinary bladder cancers, with a wide range of reported protein overexpression (0–80%) (8,9). Similar findings of 41.3% and 31% were reported in Germany and Egypt,

respectively, using immunohistochemistry by Kruger et al. (8) and Olfat et al.(9). Nedjedi et al. (10) in Saudi Arabia reported immunohistochemical overexpression of 24% in TCC. Likewise, Lae et al. (11) in France reported 5.1% HER2 overexpression. Higher incidences of overexpression were also reported by other authors, including Shawky et al. (12) and Gandour-Edwards et al. (13), who reported that HER2 was overexpressed in 62.5% and 71% of carcinomas from study subjects, respectively.

These differences in HER2 incidence have been attributed to several factors. They include use of small sample size, nonuniformity of laboratory procedures, especially utilization of different antibodies, different techniques of detection (PCR, FISH, IHC), and variation in the type of samples analyzed such as formalin-fixed versus fresh specimen, scoring criteria, and variation in patients' racial origin. Nedjadi et al. (10) reported a high concordance rate between HER2 protein overexpression and gene amplification using IHC and FISH. By contrast, Bellmunt et al. (14) found a low concordance rate between the two and also concluded that HER2 status varies among populations of different racial origins. Other possibilities that can affect the result of HER2 are differences in fixation and storage of tissue.

Importantly, HER2/neu overexpression in this study correlated significantly with grade ($p \leq 001$). This observation is consistent with several previous reports. Moch et al. (15) reported overexpression in 59% of his subjects with an increase in frequency in high-grade tumors; similarly, Moriyama et al. (16) found that the HER2 gene product was more frequently expressed in high-grade tumors. Furthermore, Aurora et al. (17) reported a significant correlation between HER2 overexpression and differentiation grade of tumor. This association is important as it an indication that HER2 can serve as a potential marker for identification of the tumor cell aggressiveness, malignant subgroup, and tumor metastatic potential. In addition, overexpression has also been associated with an advanced clinical stage and serves as an independent prognostic factor (17,18).

Overexpression of HER2 was more frequent in the fourth to sixth decades of life in this study. However, no statistically significant difference was observed between HER2 positivity and age of the study subjects ($p \leq 0.211$). This is consistent with previous studies in Egypt, Germany, France, and Saudi Arabia (12,19,2).

HER2/neu overexpression was most frequent in TCC followed by SCC while no overexpression was seen in AC. However, this association does not reach statistical significance ($p=0.208$). This is in contrast to the finding by El Gehani et al. (20), who reported a statistically significant difference between a higher expression of HER2 protein in TCC and AC compared with SCC. By contrast, Shawky et al. (16) and Eissa et al.(19) reported a higher expression in SCC but with no significant differences between the two groups. These discordant results may reflect inconsistencies of sample size in all of these studies.

Similarly, no significant association was observed between subjects with and without morphologically identified schistosomal ova. This is similar to the findings documented by Eissa et al.(19) and Shawky et al.(12) but in contrast, Aurora et al.(17) have reported a significant association between schistosomal and non-schistosomal TCC.

Conclusion

This study concludes that HER2 overexpression is seen in 40% of the study subjects with a statistically significant association between HER2 overexpression and increasing tumor grade. No significant association was observed with various histological subtypes and schistosomal comorbidity.

This review is not free from the intrinsic limitations of retrospective hospital-based studies. The study was conducted over a period of just 2years and within a single tertiary hospital. Multicentre studies with a larger sample size that allows for further elaboration of the pattern of overexpression of HER2 in urothelial carcinomas are recommended.

Author contributions

MSH led in the conceptualization and writing of the first draft. All other authors contributed equally to reviewing and editing the original draft.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
2. Kramer C, Klasmeyer K, Bojar H, et al. Heparin-binding epidermal growth factor-like growth factor isoforms and epidermal growth factor receptor/ErbB1 expression in bladder cancer and their relation to clinical outcome. Cancer 2007;109:2016–24.
3. Yusuf I, Atanda AT, Umar AB, et al. Cancer in Kano, Northwestern Nigeria: A 10-year update of the Kano cancer registry. Ann Trop Pathol. 2017;8:87–93.
4. Moussaid Y, Chargari C, Mansouri M, et al. Trastuzumab therapy in metastatic bladder carcinoma: The proof of concept. J Cancer Res Ther. 2014;2:128–31.
5. Hansel DE, Swain E, Dreicer R, et al. HER2 overexpression and amplification in urothelial carcinoma of the bladder is associated with MYC coamplification in a subset of cases. Am J Clin Pathol. 2008;130:274–81.
6. Dayalu SLN, Shashi S, Amitabha R, et al. Epidermal growth factor receptor expression in urinary bladder cancer. Indian J Urol. 2011;27:208–14.
7. Bolenz C, Shariat SF, Karakiewicz PI, et al. Human epidermal growth factor receptor 2 expression status provides independent prognostic information in patients with urothelial carcinoma of the urinary bladder. BJU international. 2010;106:1216-22.
8. Krüger S, Weitsch G, Büttner H, et al. HER2 overexpression in muscle-invasive urothelial carcinoma of the bladder: prognostic implications, Int J Cancer 2002;102:514–18.
9. Olfat H, Hani HN, Maha M, et al. The clinical significance of HER2 protein amplification/expression in urinary bladder lesion. Arab J Urol. 2015;13:146–152.
10. Nedjadi T, Jaudali A, Maurad A, et al. Prognostic value of Her2 status in bladder transitional cell carcinoma revealed by both IHC and BDISH techniques. BMC Cancer 2016;16:653.
11. Laé M, Couturier J, Oudard S, et al. Assessing HER2 gene amplification as a potential target for therapy in invasive urothelial bladder cancer with a standardized methodology: results in 1005 patients. Ann Oncol. 2010,21:815–19.
12. Shawky AEA, Hisham A, Elosaily G, et al.Her2/Neu overexpression in invasive bladder carcinoma among a cohort of Egyptian patients. World J Nephrol Urol. 2013;2:70–75.
13. Gandour-Edwards R, Lara Jr PN, Folkins AK, et al. Does HER2/neuexpression provide prognostic information in patients with advanced urothelial carcinoma? Cancer 2002;95:1009–15.
14. Bellmunt J, Werner L, Bamias A, et al. Her2 as a target in invasive urothelial carcinoma. Cancer Med. 2015;4:844–52.
15. Moch H, Sauter G, Moore D. p53 and erbB-2 protein overexpression are associated with early invasion and metastasis in bladder cancer.Virchows Arch A Pathol Anat Histopathol. 1993;423:329–34.
16. Moriyama M, Akiyama T, Yamamoto T, et al. Expression of C-ERBB-2 gene product in urinary bladder cancer. J Urol. 1991;145:423–27.
17. Aurora A, Flavia B, Delia EZ, et al. Clinical significance of Her2/neu overexpression in urothelial carcinomas. Romanian J Morpho Embry. 2010;5:277–82.
18. Sato K, Moriyama M, Mori S, et al. An immunohistologic evaluation of c-erbB-2 gene product in patients with urinary bladder carcinoma. Cancer 1992;70:2493–98.
19. Eissa S, Ali HS, Al Tonsi AH, et al. Her2/neu expression in bladder cancer: relationship to cell cycle kinetics. Clin Biochem. 2005;38:142–48.
20. El Gehani K, Al-Kikhia L, Emaetig F, et al.Overexpression of Her 2 is associated with the stage in carcinomas of the urinary bladder. Libyan J Med. 2012;7:14694