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Original Research Article **Pathology**

Immunohistochemical Study of HER-2/NEU Expression in Urothelial Bladder Carcinoma and its Correlation with Histopathological Grade

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Abstract

***Background*:** Human epidermal growth factor receptor 2 (HER2) overexpression and amplification have been considered as a therapeutic and prognostic target in a quantity of tumors although conflicting data exist about the incidence and clinical consequence of HER2/neu status in the patients. The growth risk of bladder cancer is age dependent. In general, adolescent and young adults tend to develop well differentiated non-invasive, rather than invasive bladder cancer. ***Objectives*:** The aim of this study is to assess the Immunohistochemical study of HER-2/NEU Expression in urothelial bladder carcinoma and its correction with histopathological Grade. ***Methods*:** This was a cross sectional study. Patients with paraffin blocks of urinary bladder tissue which were histologically diagnosed as urothelial carcinoma at the Department of Pathology, BIRDEM and other private laboratories were included in our study during the period of January 2016 to December 2018. ***Results*:** This study shows that, a total of 56 histologically diagnosed cases of urothelial carcinoma were included. There were 34 cases (60.7%) of high grade and 22 cases (39.3%) of low-grade urothelial carcinomas. The age of the patient ranged from 23 years to 98 years (mean 66.34±15.3 years). Majority of patients (50.98 %) belonged to 61 to 80 years age group. High grade tumors were predominantly seen at an older age. There were 42 males (75.0%) and 14 females (25.0 %) with a male to female ratio of 3:1. ***Conclusions*:** Significant correlation in expression of HER-2/Neu was found with grading of urothelial carcinoma. A positive HER-2 status is associated with aggressive urothelial carcinoma and provides independent prognostic information for urothelial carcinoma recurrence and mortality.

Keywords: Human epidermal growth factor receptor 2 (HER2); Therapeutic; Prognostic; Adolescent; Bladder cancer.

INTRODUCTION

The urinary bladder cancer was the second common malignancy (10.4%) in Egypt. It is the most frequent cancer among males (16.2%) and fifth common among females (4.4%), which is strikingly higher than most other parts of the world [1]. According to the American Cancer Society Statistics, about 74690 new cases of UBC are expected to occur in the United States in 2014. In Iraq the incidence of bladder cancer has increased sharply with most types of cancer in last few years due to exposure to war pollution [2].

Bladder is an organ of urinary system which act as a temporary reservoir of urine. The size, shape, position and relations of bladder vary according to its content and the state of neighbouring viscera. It lies entirely in the lesser pelvis when the bladder is empty but expands anterosuperiorly into the abdominal cavity during distension. An empty bladder is tetrahedral in shape and divided into the following portions: superior surface or dome, posterior surface or base, neck, apex and two inferolateral surfaces [3].

The superior surface faces superiorly and is covered by the pelvic peritoneum. The posterior surface

or base is triangular and located posteroinferiorly. It is separated from the rectum by the uterine cervix and the proximal portions of the vagina in females and by the seminal vesicles and the vasa deferential in males. The apex lies anterosuperiorly [4].

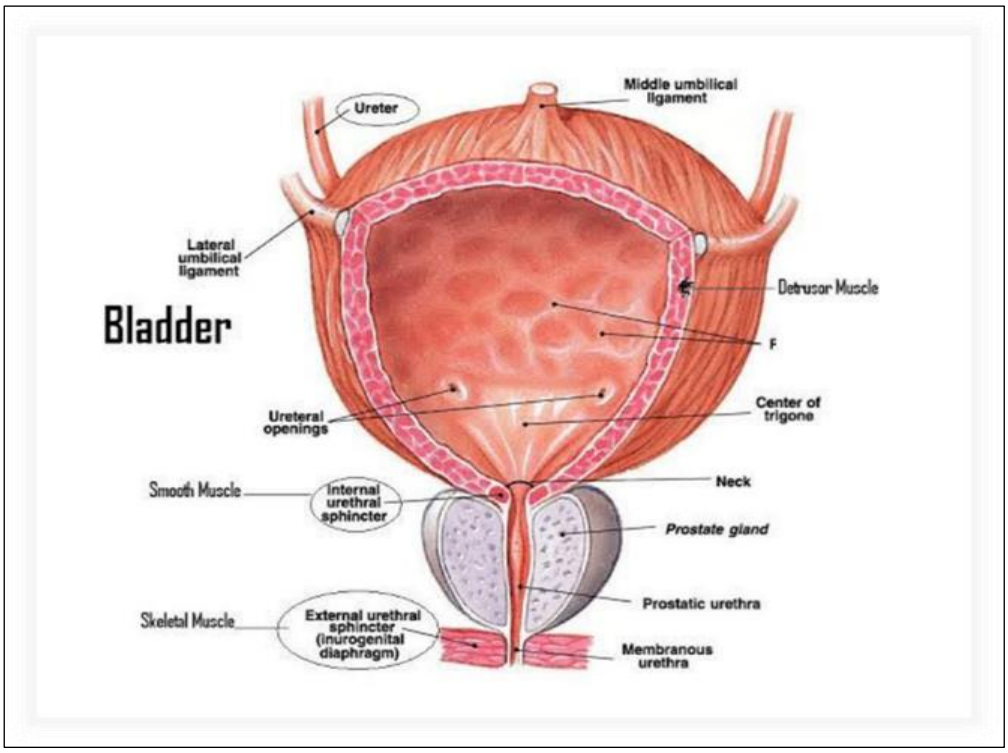

Figure 1: Anatomy of Urinary Bladder

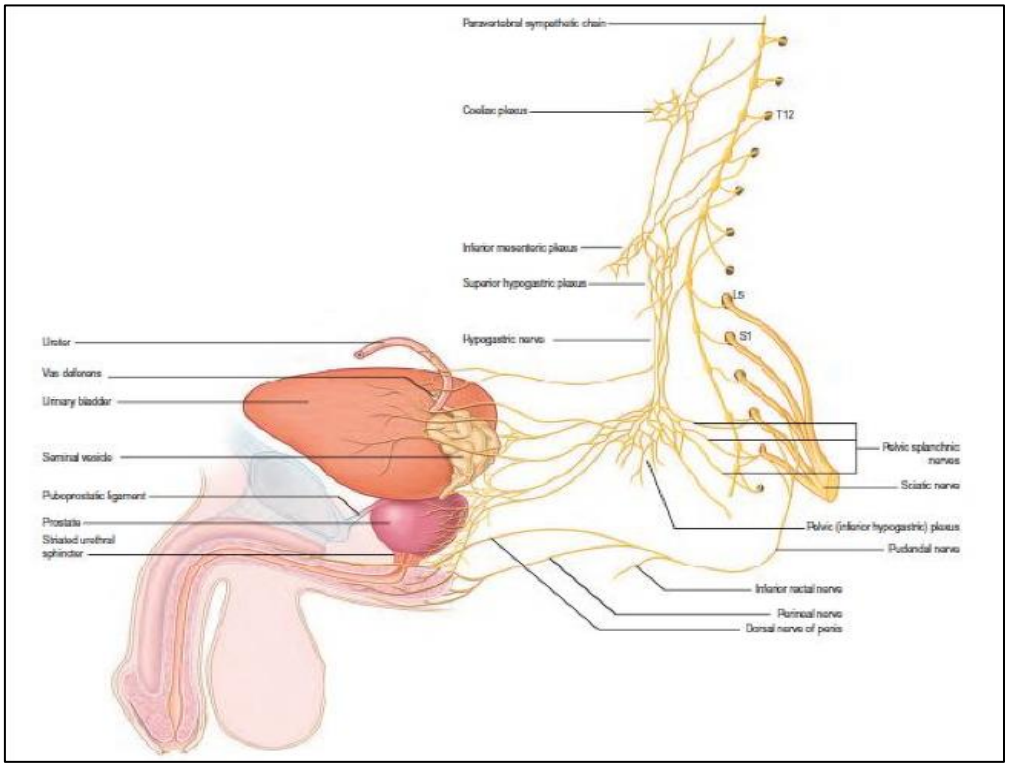

Figure 2: Innervation of the lower urinary tract and male genitalia (Standring *et al.*, 2016).
Source: *www.internet.tdmu.edu.ua* (Accessed on 2019 January27)

The urinary bladder is supplied by both sympathetic and parasympathetic nerves. The sympathetic supply derived from T_{11} through L_2 nerves. The parasympathetic nerves come from S_2 through S_4

and travel to the bladder via the pelvic splanchnic nerve and inferior hypogastric plexus [5]. Urinary bladder cancer is the ninth most common malignancy worldwide. It represents the fourth most common cancer in men and the eight in women. The incidence of urinary bladder carcinoma varies over the world with highest rate in developed countries [6]. Incidence rates are high in many southern and eastern European countries, North America and the Middle East [7] and Lymphatics which drain the bladder begin in mucosal, intermuscular and serosal plexuses. There are three sets of collecting vessels. Vessels from the superolateral aspects of the bladder dran into the external iliac lymph nodes. Vessels from the neck and fundus drain into the internal iliac lymph nodes and some into the sacral or common iliac lymph nodes [8].

The bladder is supplied principally by the branches of the anterior trunk of the internal iliac arteries. The superior vesicle arteries supply the anterior and superior aspects of the bladder. The inferior vesicle arteries supply the bladder base. It is supplemented by the obturator and inferior gluteal arteries. In the female, additional branches are derived from the uterine and vaginal arteries [9]. The veins of the urinary bladder form the vesical venous plexuses and drain into the internal iliac veins. In the male, this plexus envelops the bladder base, prostate, and seminal vesicles and connects with the prostatic venous plexus. In females, it covers the bladder neck and urethra and communicates with the vaginal plexus [10]. New cases of urinary bladder carcinoma were 549,393 in 2018 which were 3% of all cancers. Out of which 199,922 deaths were estimated in the same year [11]. Urothelial carcinoma (also known as transitional cell carcinoma or “TCC”) comprises approximately 90% of all primary tumors of the bladder.

METHODS

This was a cross sectional study. Patients with paraffin blocks of urinary bladder tissue which were histologically diagnosed as urothelial carcinoma at the Department of Pathology, BIRDEM were included in our study during the period of January 2016 to December 2018.

Some paraffin blocks were also collected from Department of Pathology, BSMMU and other private laboratories. Tissues collected from all age groups and both sexes, who had undergone transurethral resection of bladder tumor (TURBT) or cystectomy, were included.

Statistical evaluation of the results used to be got via the use of a window-based computer software program devised with Statistical Packages for Social Sciences (SPSS-24).

RESULTS

Table I: Distribution of the study cases according to age (n=56)

Age (Year)	n	%
20-29	1	1.8
30-39	1	1.8
40-49	5	8.9
50-59	9	16.1
60-69	17	30.4
70-79	12	21.4
>80	11	19.6
Mean±SD	66.34±15.3	
Range (Min-Max)	23-98	

Table I demonstrated the age of 56 Patients aged 20 to >80 years. Here according to Age distribution, 1(1.8%) were 20-29, 1(1.8%) were 30-39, 5(8.9%) were 40-49, 9(16.1%) were 50-59, 17(30.4%) were 60-69, 12(21.4%) were 70- 79 and 11(19.6%) were >80.

Figure I Distribution of cases by sex (n=56).

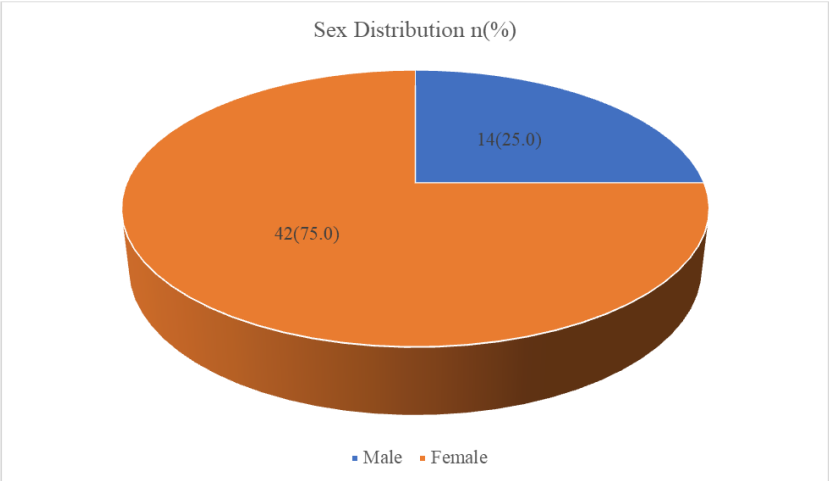

Figure I: Pie chart showing sex of the study patients

Figure I show the distribution of cases according to sex. 42 (75.0%) patients were male and 14 (25.0%) were female. The male to female ratio was 3.1.

Figure II Sex distribution of Urothelial carcinoma in different age group (n=56).

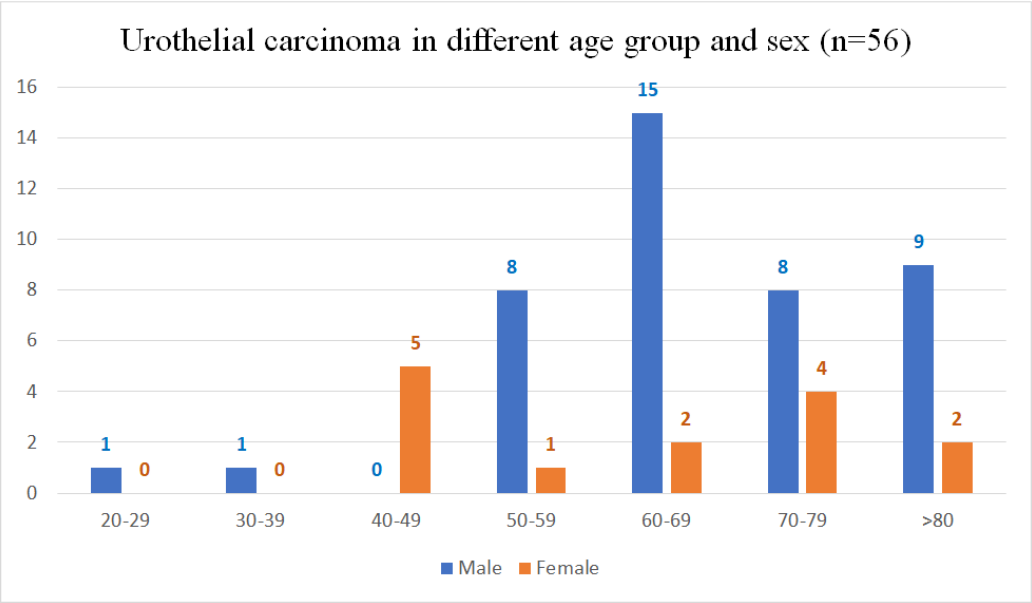

Figure II: Bar diagram showing distribution of sex in different age group

Figure II shows the distribution of sex according to age. The incidence of urothelial carcinoma was higher in males of all age groups. Females were more affected between 40-49 years.

Figure III Prevalence of histological grades of urothelial carcinoma in different age groups (n=56).

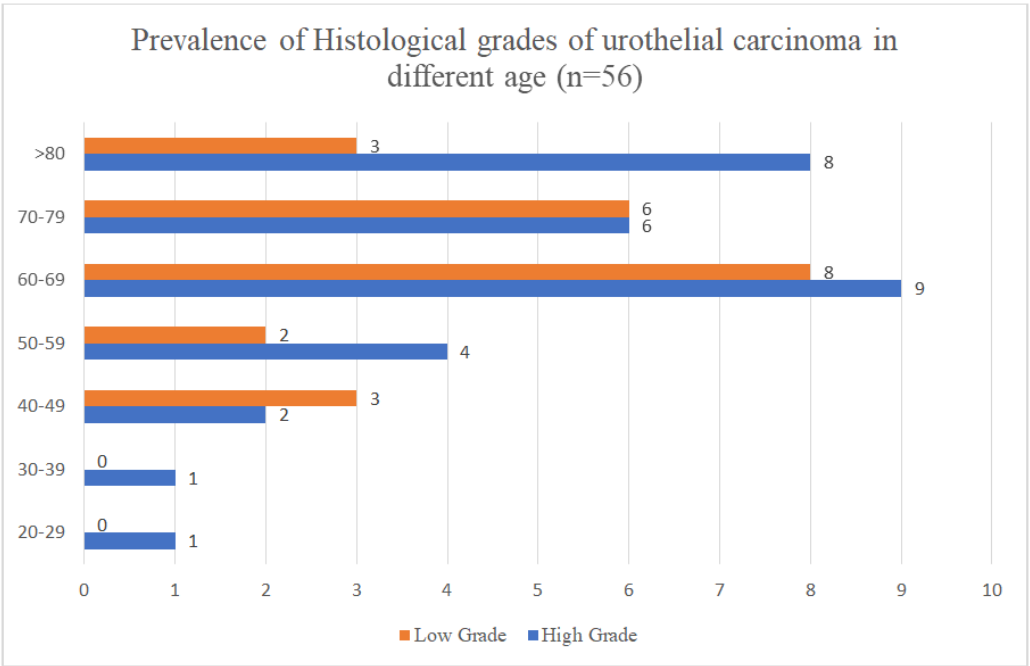

Figure III: Bar diagram showing prevalence of histological grades of urothelial carcinoma in different age group

Figure III shows the prevalence of histological grades in differents age group. High grade urothelial carcinoma occurred more or equally in age except in 40-49 age group where low grade urothelial carcinoma was more common.

Figure IV Histological grades of urothelial carcinoma (n=56).

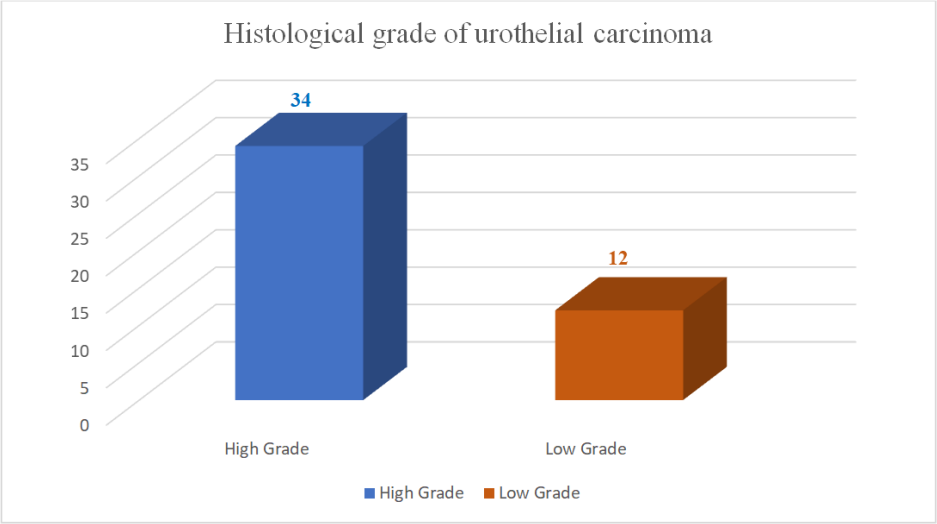

Figure IV: Bar diagram showing histological of urothelial carcinoma

Figure IV shows the distribution of the study patients by histological diagonosis. It was observed that high grade urothelial carcinoma was more prevalent than low grade urothelial carcinoma.

Table II: Association between over-expression of HER-2 and its scoring with histological grades of urothelial carcinoma (n=56)

Interpretation	Histological diagnosis				P value
	High Grade (n=34)		Low Grade (n=22)		
	n	%	n	%	
Positive	25	78.78	7	21.22	0.001
2+	16	47.1	2	9.1	
3+	10	29.4	5	22.7	
Negative	8	23.5	15	68.2	
0	7	20.6	13	59.1	
1+	1	2.9	2	9.1	

Table II demonstrated the Association between over-expression of HER-2 and its scoring with histological grades of urothelial carcinoma (n=56). Immunohistochemical expression of HER-2 was observed in 26 (78.8%) cases of high grade and 7 (21.22%) cases of low-grade urothelial carcinoma. The difference was statistically significant ($p<0.05$). HER-2 expression was scored as per ASCO where 0 and 1+ were considered as negative and a score of 2+ and 3+ were considered as positive.

Figure 3: (Case no-1): Photomicrograph of a high grade urothelial carcinoma (HE10x)

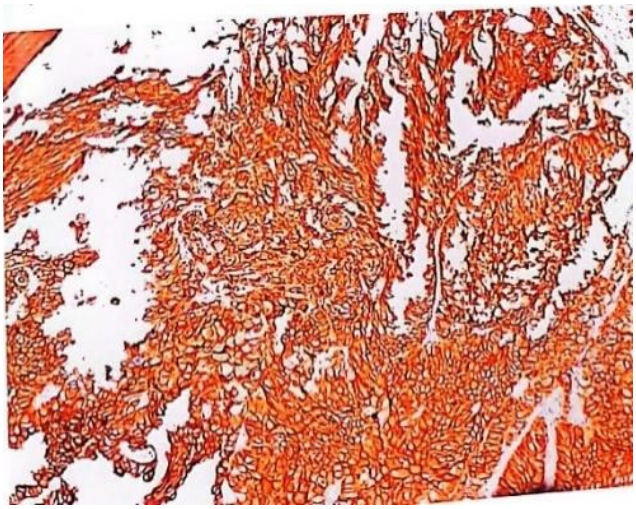

Figure 4: (Case no-1): photomicrograph of a high grade urothelial carcinoma showing 3+ staining of HER-2/Neu (IHC 10x)

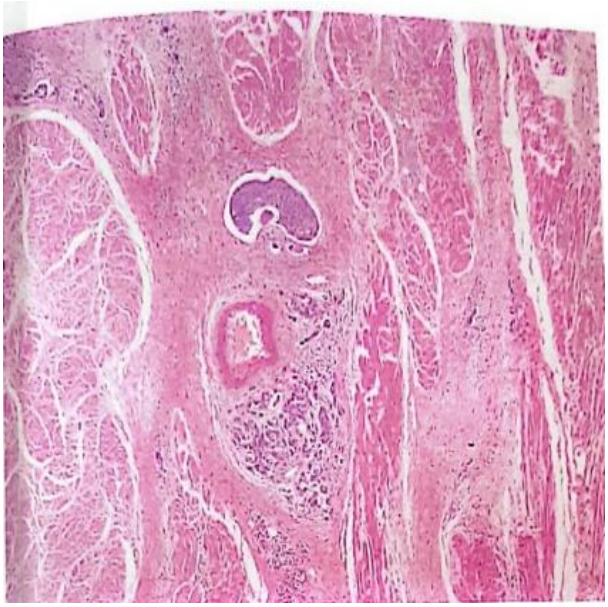

Figure 5: Case no-24): photomicrograph of a high grade muscle invasive urothelial carcinoma (HE10x)

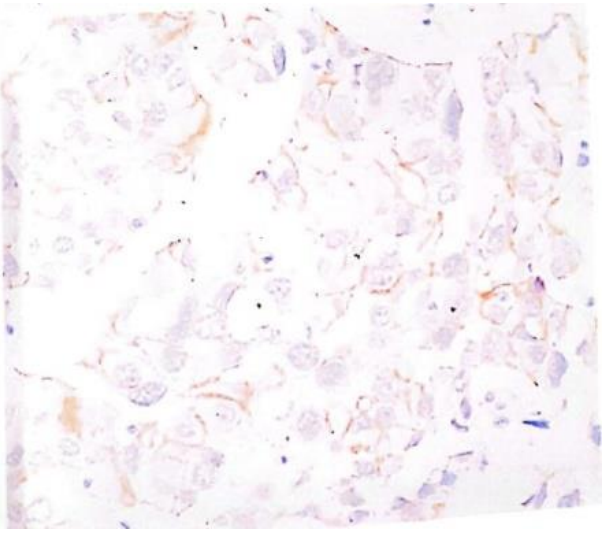

Figure 6: (Case no-24): Photomicrograph of a high muscle invasive urothelial carcinoma showing3+ staining of HER-2/Neu (IHC40x)

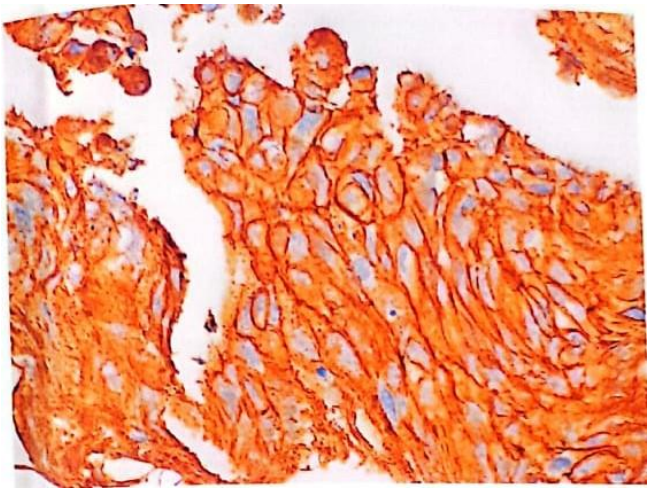

Figure 7: (Case no-36): Photomicrograph of a high grade urothelial carcinoma showing 3+ staining of HER-2/Neu (IHC 40x)

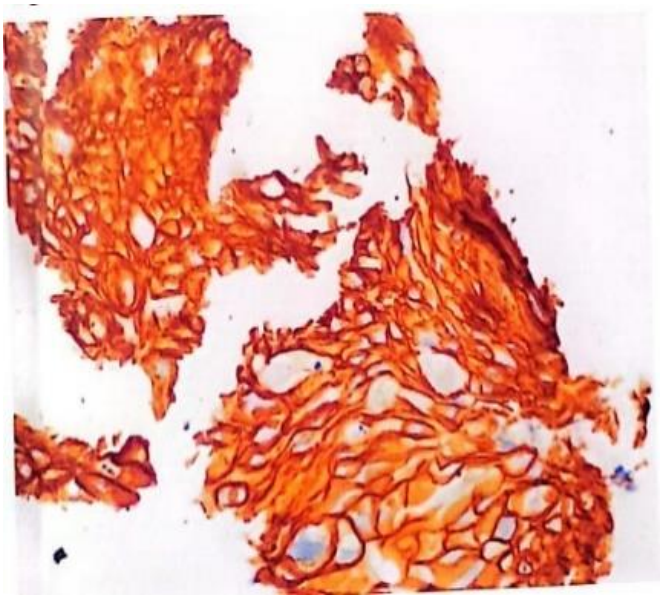

Figure 8: (Case no-38): Photomicrograph of a high grade urothelial carcinoma showing 3+ staining of HER-2/Neu (IHC 40x)

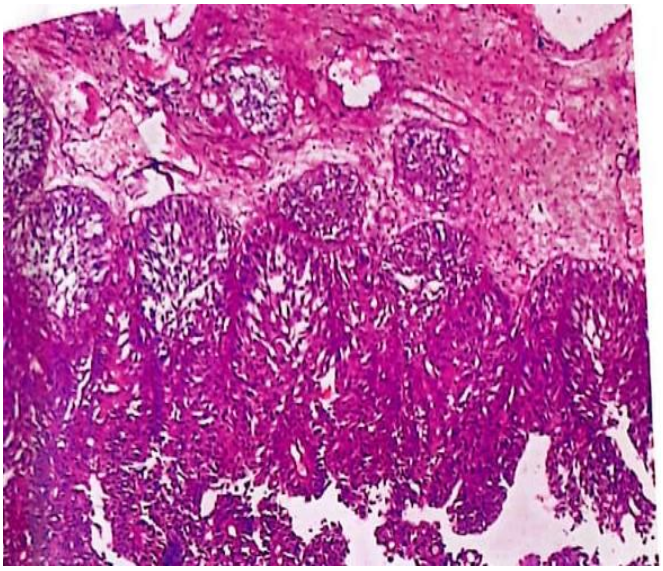

Figure 9: (Case no-25): Photomicrograph of a low grade urothelial carcinoma HER-2/New (HE 10x)

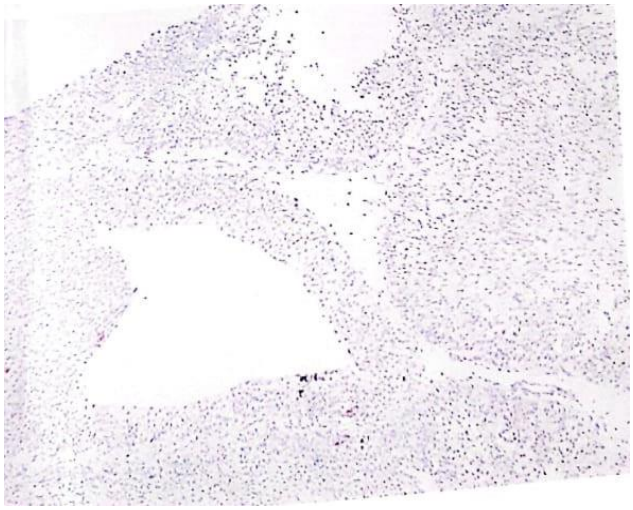

Figure 10: (Case no-25): Photomicrograph of a low grade urothelial carcinoma showing negative staining of (IHC 10x)

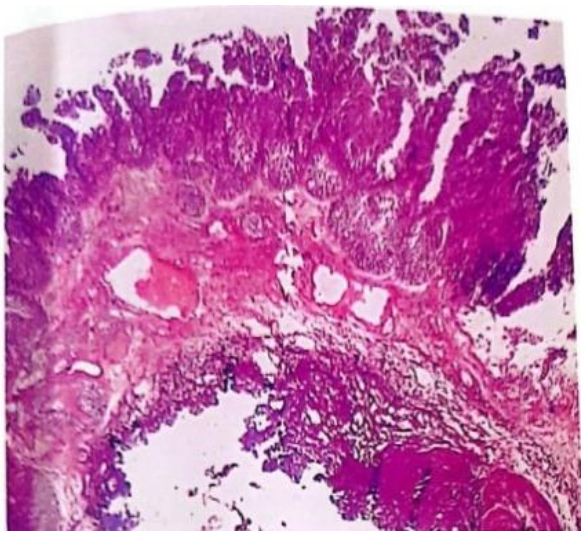

Figure 11: (Case no-48): Photomicrograph of a low grade urothelial carcinoma (HE 4x)

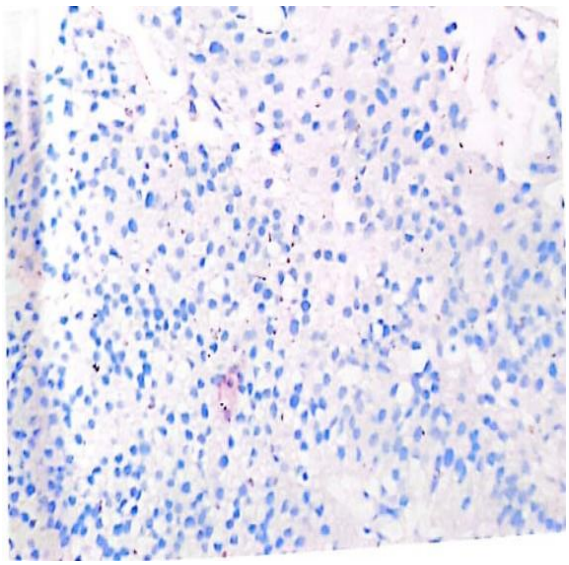

Figure 12: (Case no-48): Photomicrograph of a low grade urothelial carcinoma showing 1+ staining of HER-2/Neu (IHC 40x)

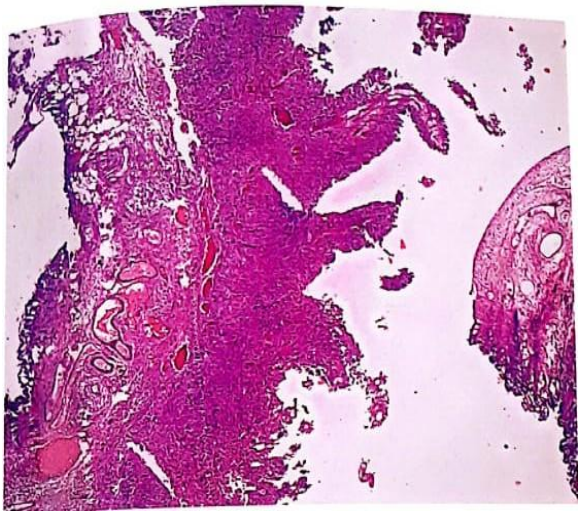

Figure 13: (Case no-42): Photomicrograph of a high grade muscle invasive urothelial carcinoma (HE 4x)

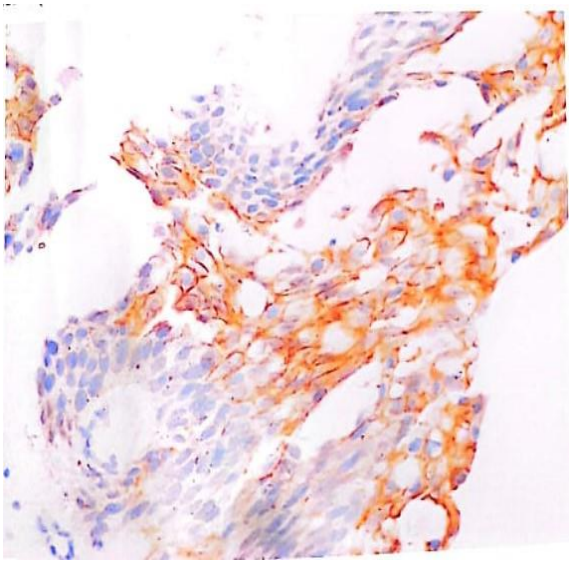

Figure 14: (Case no-42): Photomicrograph of a high grade muscle invasive urothelial carcinoma showing 3+ staining of HER-2/Neu (IHC 40x)

Figure 15: (Case no-50): Photomicrograph of a low grade urothelial carcinoma (HE 4x)

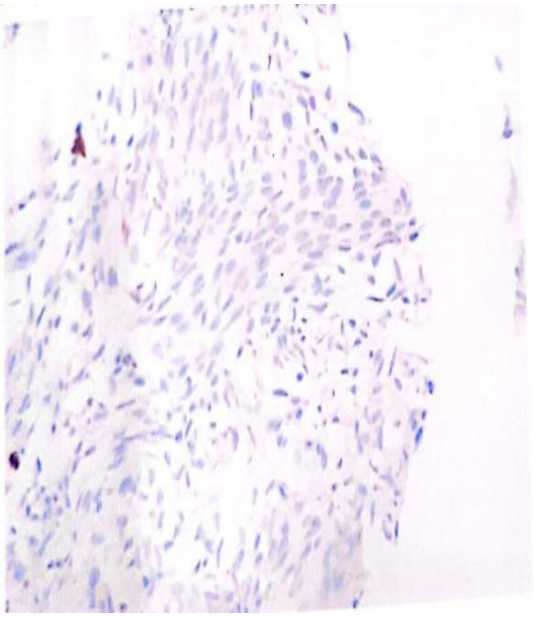

Figure 16: (Cases no-50): Photomicrograph of a low grade urothelial carcinoma showing 2+ staining of HER-2/Neu (IHC 40x)

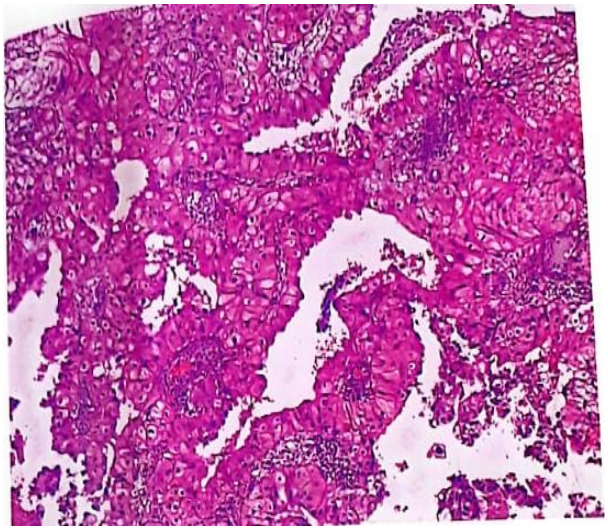

Figure 17: (case no-11): Photomicrograph of a high grade urothelial carcinoma (HE 10x)

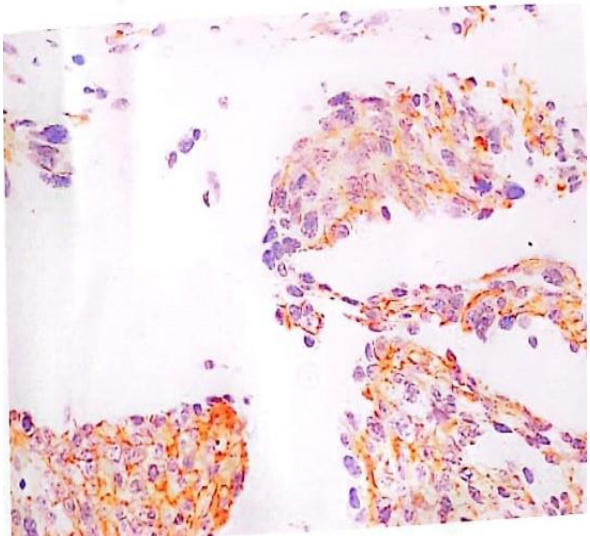

Figure 18: (Case no-11): Photomicrograph of a high grade urothelial carcinoma showing 3+ staining of HER-2/Neu (IHC 40x)

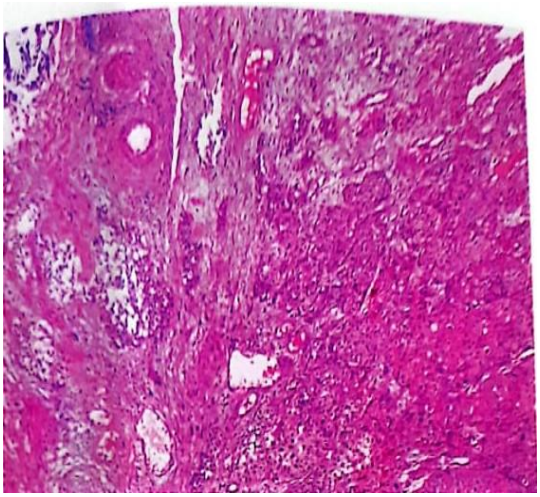

Figure 19: (Case n o-34): Photomicrograph of a high grade urothelial carcinoma showing 3+ staining of HER-2/Neu (IHC 40x)

DISCUSSION

Bladder cancers are associated with several molecular alterations and complex biological pathways that regulate cellular processes, such as proliferation, differentiation, angiogenesis, metastasis and apoptosis. The discovery of new biological markers, may lead to the improvement of clinical prediction and modify the therapeutic approaches of bladder cancer patients with the purpose of reducing the risk of progression [12]. Many studies have concentrated on the evaluation of biological markers for bladder cancer. Among them the human epidermal growth factor receptors represent promising therapeutic targets [13, 14].

This cross-sectional study was carried out with an aim to detect the immunohistochemical expression of HER- 2/Neu in urothelial bladder carcinoma and to correlate its expression with histopathological grade. A total 56 cases of urothelial carcinoma from Pathology Department of BIRDEM and other centers from Dhaka city during June 2017 to May 2019 were included in this study. Only the urothelial carcinoma of low grade and high grade were included. Other types of urothelial tumors were excluded from the study.

In present study, maximum patients were in age group 60-69 years (30.4%). Mean age of the patient was 66.34±15.3 years with a range of 23 years to 98 years. In this study, there were 42(75.0%) males and 14(25.0%) females with a male to female ratio 3:1. This result is consistent with those mentioned by Xiefeng *et al.,* 2008, Parkin *et al.,* 2008 and Gehani *et al.,* 2014 where they found a male to female ratio of 3.2:1, 3.63:1 and 3:1 respectively.

The majority 51(91.1%) of the cases included in this study were obtained by TURBT. All the cases were removed surgically by TURBT which is comparable with the current study. In this current study, there were 34(60.7%) cases of high grade and 22(39.3%) cases of low-grade urothelial carcinomas.

The association between age and histological grades of TCC was evaluated. High grade tumors were predominantly seen in older age. This reflects aggressive tumor behavior in older patients. This result was supported by the study of Ramesh *et al.,* (2016) who found high grade cancers predominantly in patient above 60 years of age [15].

This study evaluated the association between over-expression of HER-2 and its scoring with histological grades of TCC. Immunohistochemical over-expression of HER-2/Neu was observed in 26(76.5%) cases of high grade and 7(31.82%) cases of low-grade urothelial carcinoma. The difference was statistically significant ($p<0.05$). Omran *et al.,* (2012) found HER-2 expression in 59% of bladder carcinomas and also proved that the staining intensity for the high-grade group was higher than that of the low-grade group [16]. Shawky *et al.,* in 2013 revealed 92.3% of high grade and 14.2% of low-grade urothelial carcinomas for HER-2 over-expression and a strong correlation with histologic grade in 32 cases of urothelial carcinomas. These results are similar to the present study [17].

Jawad *et al.,* (2016) found a significant correlation between expression of Her-2/Neu and the WHO 2004 grading system of urothelial carcinoma [18]. There were 20/25 (80%) of high-grade carcinoma cases showing positivity to immunohistochemical expression of HER-2/Neu marker, while 9/20 (45%) of low-grade cases were positive for HER- 2/Neu marker. A significant correlation was found between HER-2 over-expression and tumor grade ($p=0.003$) [19, 20]. These findings support the results of the current study. However, opposite result was seen in a study done by Rafael *et al.,* (2006) [21]. They did not find any significant association ($P> 0.05$) between HER-2 expression and histologic grades.

So, assessment of HER-2 status can be done to identify patients at high risk of disease progression who

may benefit from adjuvant HER-2 targeted mono or combined therapy.

CONCLUSION

Significant correlation in expression of HER-2/Neu was found with grading of urothelial carcinoma. A positive HER-2 status is associated with aggressive urothelial carcinoma and provides independent prognostic information for urothelial carcinoma recurrence and mortality. So, patients with HER-2 positive urothelial carcinoma could potentially be benefited from adjuvant HER-2 targeted monotherapy or combined therapy.

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