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Review Article

Clinico-Pathological Correlation of Urothelial Carcinoma of Urinary Bladder with P63 and CK20 Immunostain

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Abstract: Urinary bladder carcinoma is a common cancer in the Indian population in increasing incidence over the past few decades. Most of the tumours of the urinary bladder are urothelial carcinomas, comprising approximately 90% of all primary tumours of this organ. Urothelial carcinoma is one of the major causes of morbidity and mortality throughout the world and also in India. The objective is to assess the epidemiological assay of urothelial carcinoma of the bladder and to correlate with clinical and pathological findings, and also to help in accurate diagnosis with respect to grade and invasiveness by using histomorphology and immune markers, thus guiding the treatment protocol. The study was conducted in the Department of Pathology in association with the Department of Urology, IPGME&R, Kolkata, for a duration of 2 years. A total of 100 cases were selected with cystoscopically evident tumour and undergoing Trans Urethral Resection of Bladder Tumour (TURBT). The biopsy samples was collected and further processed in the Department of Pathology. Haematoxylin-eosin and immunostaining with CK20 and p63 were done. Data was analysed by Statistica version 6 Software [Tulsa, Oklahoma: StatSoft Inc., 2001. Fisher's exact test and Chi square test were conducted to assess relation between categorical variables. Results were considered significant at $P<0.05$. A total of 100 cases were studied. Most cases were males and in the elderly age group. Most common presenting symptom was haematuria. Smoking history was present in 62% cases. Histologically, 90% cases were Papillary type and invasive at the time of presentation. Most of the invasive cases were of High Grade. The stage and grade of urothelial carcinoma correlated with the extent of CK20 and P63 immunoexpression ($p<0.05$). In this study in a tertiary care hospital we have studied the relation between different clinical and histological parameters in cases of urinary bladder carcinomas using TURBT specimens.
Keywords: Urothelial Carcinoma, Clinicopathological, CK20, P63

INTRODUCTION

Urinary bladder cancer ranks ninth in worldwide cancer incidence (approximately 356,000 new cases are detected each year) and it is the seventh most common malignancy in men and 17th in women [1]. Furthermore, approximately 145,000 patients die from this disease worldwide per year [2].

In India, according to the recent reports of the National Cancer Registry Programme, the overall incidence rate of the urinary bladder cancer is 2.25% (per 100,000 annually): 3.67% among males and 0.83% for females [3].

Urothelial cancer predominantly affects male patients over 50 years of age, being rare in children. The average age at diagnosis is 60.2+/- 4.4 years [4]. Male: Female ratio is 3:1. Among the newly diagnosed bladder cancers, 40-45% are high-grade lesions and more than half of these are muscle invasive at the time of diagnosis.

A number of factors have been known to increase the risk of this cancer, like cigarette smoking, occupational exposure [5] to chemicals like aromatic amines or aniline dyes [6] or diesel exhaust, petroleum

products, leather tanning, textile work, auto work, truck driving and plumbing.

Patients classically present with painless gross haematuria seen in 80-90% of patients. Cystoscopy, cytology, and biopsy are the principal diagnostic tests.

Histologically, almost all bladder cancers originate in the urothelium. Worldwide, morphologically, 90% of the bladder tumours are transitional cell carcinoma [7]. Squamous cell carcinoma is the second most common type and 2% of bladder cancers are adenocarcinomas.

The World Health Organisation (WHO) has recommended that urothelial tumours be classified as muscle-invasive and non-muscle-invasive with Tumour-Node-Metastasis staging along with grading of tumour based on morphology.

OBJECTIVES

To assess the epidemiological assay of urothelial carcinoma and to correlate with clinical and pathological findings, and also to help in accurate diagnosis with respect to grade and invasiveness by using histomorphology and immune markers, thus guiding the treatment protocol.

MATERIALS AND METHODS

The study was conducted in the Department of Pathology, IPGME&R, Kolkata, in association with the Department of Urology, IPGME&R, Kolkata within a duration of 2 years. It was done in patients >15 years and attending the Department of Urology, with features of hematuria and pain abdomen with cystoscopically evident tumour and undergoing Trans Urethral Resection of Bladder Tumour (TURBT). After processing tissue, slides were prepared and stained with H&E. Immunostaining with CK20 (Novocastra Mouse Monoclonal Antibody Cytokeratin 20, RTU-CK20, Clone- Ks20.8) and P63 (Dako P63 Protein Mouse Monoclonal Anti-Human Antibody, M7317, IR662, Clone- DAK-p63) was done. Semiquantitatve score was used to record results of CK20 staining, Neegative for <5% of tumour cells, 1+ for 6-25% of tumour cells, 2+ for 26-50% of tumour cells and 3+ for >50% of tumour cells. p63 was also scored semiquantitatively as 0 or Negative for 0% tumour cell positivity, 1+ or focal for 1-10% positivity, 2+ or moderate for 11-50%, and 3+ or diffuse for >50% positivity.

STATISTICAL ANALYSIS

Data was analysed by Statistica version 6 Software [Tulsa, Oklahoma: StatSoft Inc., 2001. Fisher's exact test and Chi square test were conducted to assess relation between categorical variables. Results were considered significant at $P<0.05$.

RESULTS

In this study of 100 cases 86% were males, while 14% were females and the male to female ratio was around 6.1:1. Most of the study cases were of elderly age group in the 61-80 years'age group (n=46), followed by 41-60 year age group (n=40). . The average age of presentation in this study was 58 years. There was 75% distribution among Hindus and 25% among Muslims. Haematuria with or without other symptoms, was the most common presenting complaint (73%), while rest complained of symptoms other than hematuria like pain abdomen or dysuria. Most of the cases studied were smokers (62%). Histologically, 90% cases of Papillary type, 5% has squamous metaplasia, 4% had microcyst formation, and 1% had glandular differentiation. There were 51% Low Grade tumours compared to 49% High Grade tumours. Most of the cases had invasive disease (75%) at the time of presentation. Most of the invasive cases (54.67%) were of High Grade. Vice versa, most of the High Grade cases were invasive (83.67%). Stage-wise, 23% were of stage PTa, 48% were PT1 and 19% were PT2. Out of 100 cases, 13% showed 1+, 67% showed 2+, and 20% showed 3+ staining for CK20. Majority of the invasive tumours (66.67%) and noninvasive tumours (68%) showed 2+ CK20 staining. Noninvasive tumours showed 0% CK20 3+ stain while invasive tumours showed 26.67% 3+ staining. More than half of the PT2 cases (55.17%) were 3+ for CK20. Majority of the PT1 cases (85.42%) and PTa cases (65.22%) showed 2+ staining for CK20. Out of 100 cases, 28% showed 1+, 40% showed 2+, and 32% showed 3+ staining for p63. Out of the invasive tumours, 52% showed 2+ staining and 48%showed 3+ staining. Out of the noninvasive tumours, 40% showed 2+staining, 32% showed 3+ and 28% showed 1+ staining with p63. Only stage PTa showed 1+ staining (30.43%) for p63. Amongst the PT1 and PT2 cases 52.08% and 44.83% stained 3+ for p63.

DISCUSSION

Urinary bladder tumour is a very common occurrence in today's life and staging as well as grading of the tumour is of utmost importance for treatment, survival and prognosis. More than 90% of the tumours of the urinary bladder are urothelial carcinomas (Fig 1A). This study demonstrates the epidemiological distribution of urothelial carcinomas in eastern India. It also emphasises on the relation between stage, grade and ck20 and p63 immunostaining.

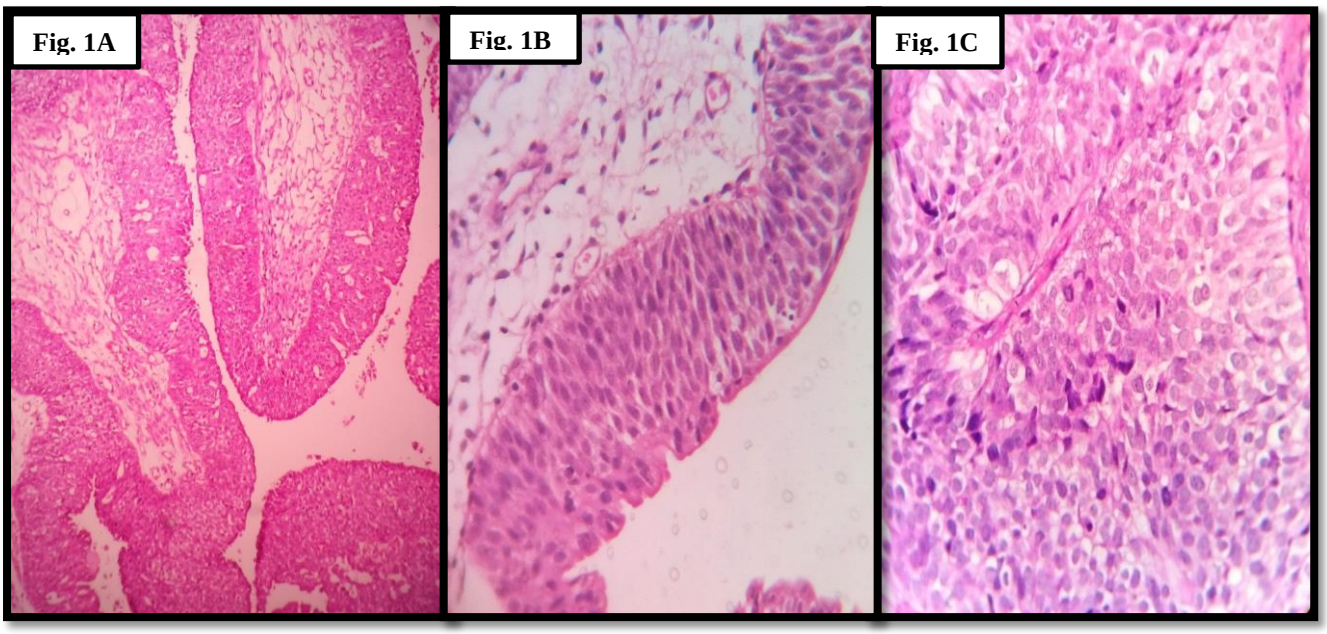

Fig-1: A) Photomicrograph showing papillary urothelial carcinoma (low magnification, 10X); B) Photomicrograph showing Low Grade urothelial carcinoma (high magnification, 40X); C) Photomicrograph showing High Grade urothelial carcinoma (high magnification, 40X)

In this study of 100 cases 86% were males, while 14% were females and the male to female ratio was around 6.1:1 which is higher than the 3:1 to 4:1 ratio worldwide [8, 9]. An Indian study done by Ranu *et al* done in West Bengal have also shown a ratio of 6:1 [7, 9]. However in Indian population the ratio is much higher being 8.6:1 [10].

Most of the study cases were of elderly age group in the 61-80 year age group, followed by 41-60 year age group which was next most common, similar to most other studies [11-13]. The average age of presentation in this study was 58 years. This was similar to a study done in South India by Panchal Jaimin and Khandige Shreesha which showed a mean age of 59.3 years [14]. However 5 of them were in their second decade. The lowest age noted was 24 years and the highest age was 90 years.

Although no study has specified religion to be associated with urothelial carcinoma, this study found 75% distribution among Hindus and 25% among Muslims. The study done by Ranu *et al*, found the tumour 5 times more common in Hindus than Muslims, compared to 3 times higher rate in our study [11]. However, both studies show greater prevalence in Hindu community.

The most common presenting symptom was hematuria which was found in 73% cases, while 23% presented with other complaints like pain abdomen and dysuria. This is in accordance with other studies like Panchal Jaimin and Khandige Shreesha (79.3%) [14], Husain *et al* [15]' (84.3%) and Rasool *et al* (78%) [16] which also showed hematuria to be the most common symptom.

A positive history of smoking was found in 62% cases, which is a known causative agent. Of the total study group, 54% were male smokers and 32% were male non-smokers. On the other hand, 8% were female smokers and 6% were female non-smokers. Study group by Ranu *et al* comprised of 75% smokers [11].

The study group comprised of people with different occupations and no specific occupation could be pointed out. However, 75% of them were either farmer (29%), labourer (21%), driver (10%) and plumber (15%). All the females were housewives. In the study by Ranu *et al* 50% were among labourer and industry workers, compared to 50% incidence in farmers and labours in our present study [11].

Histologically, tumour grading is of utmost importance. The revised WHO classification 2016 has now emphasised that all tumours be graded as High or Low Grade tumours, in addition to their invasive status. Tumours are graded according to pleomorphism, atypia, loss of polarity and mitotic figures. In this study, 51% were Low grade (Fig 1B) while 49% were High grade (Fig 1C). According to literature, most of the invasive cases are high grade. This is in accordance with the present study which shows most of the invasive cases were high grade (54.67%), while most of the noninvasive cases were low grade (68%). Similar result

was also shown in studies by Biswas *et al*, Laishram *et al* and study by Panchal Jaimin and Khandige Shreesha where most of the noninvasive tumours were low grade [13, 14, 16].

Most of the study cases were smokers. Duration and amount of smoking is equally important for development of cancer. Only those cases were considered as 'smokers' who had a history of smoking for more than 5 years. A higher percentage of Low grade tumours were seen to be smokers (68.63%) as compared to High grade tumours (55.10%). Thus it may be postulated that smoking tobacco for longer durations and higher amount of smoking may lead to progression to higher tumour grade.

Urothelial carcinomas are said to be invasive when they have invaded the lamina propria (PT1) and muscularis propria (PT2). In the present study, 75% were invasive while 25% were noninvasive. A higher percentage of high grade tumours were invasive (83.67%) compared to low grade tumours (66.67%). Laishram *et al* [13], however, studied 50% of the high grade tumours had invasion.

In this study both males and females had comparable number of invasive cases, namely, 74.42% and 78.57% respectively. Thus invasion does not seem to be predisposed to any specific gender group.

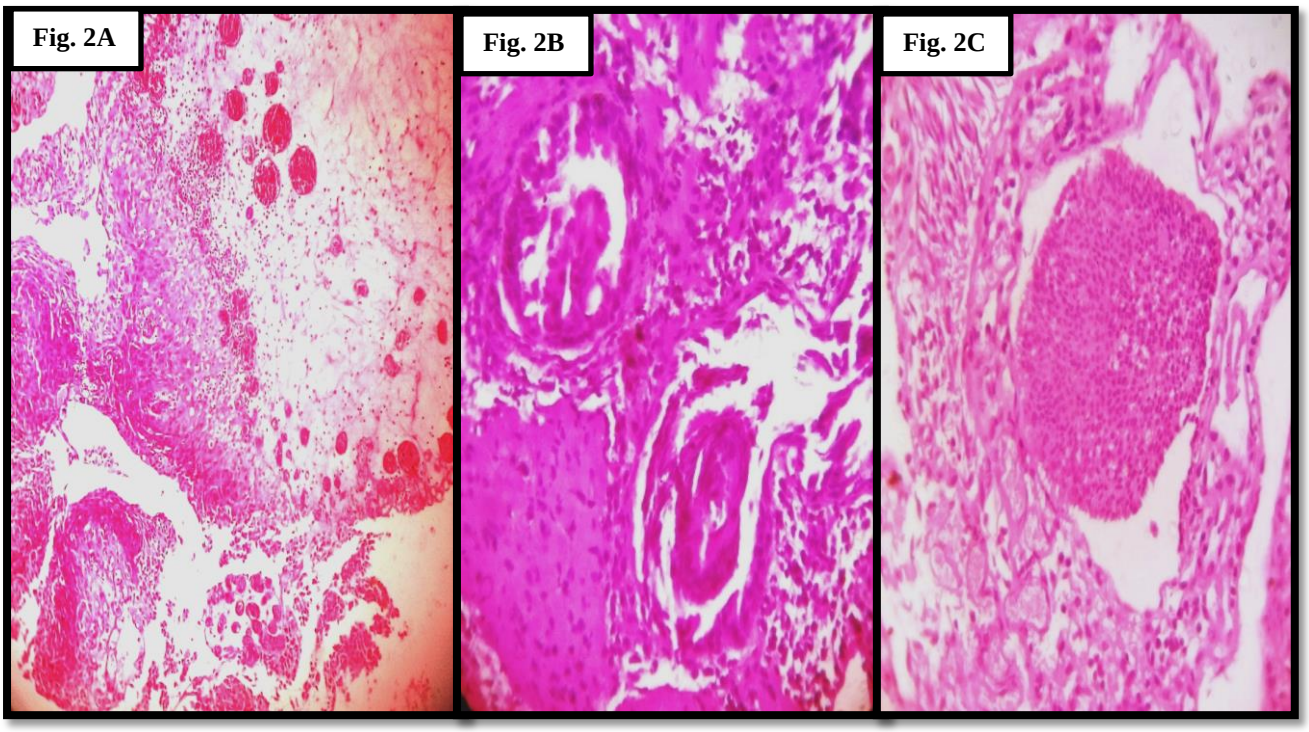

Fig-2: A) Photomicrograph showing urothelial carcinoma with invasion into lamina propria (low magnification, 10X); B) Photomicrograph showing urothelial carcinoma with invasion into muscularis propria (high magnification, 40X); C) Photomicrograph showing urothelial carcinoma with lymphovascular invasion (high magnification, 40X)

Out of 100 cases, 23% were of stage PTa, 48% were PT1 (lamina propia invasive) (Fig 2A) and only 19% were PT2 (muscle invasive) (Fig 2B). A higher percentage of muscle invasive cases were seen in studies in Pakistan by Ahmed *et al* [17] (37.6%), in SriLanka (35%) by Sasikumar *et al* [18], and in India by Laishram *et al* [13] (36.36%). The lower percentage of muscle invasive cases in our study may be attributed to earlier detection of cases due to greater awareness of the patients and higher clinical suspicion by the clinicians, thus limiting the number of cases progressing to muscle invasive disease. Lymphovascular invasion was noted in 26% cases (Fig 2C).

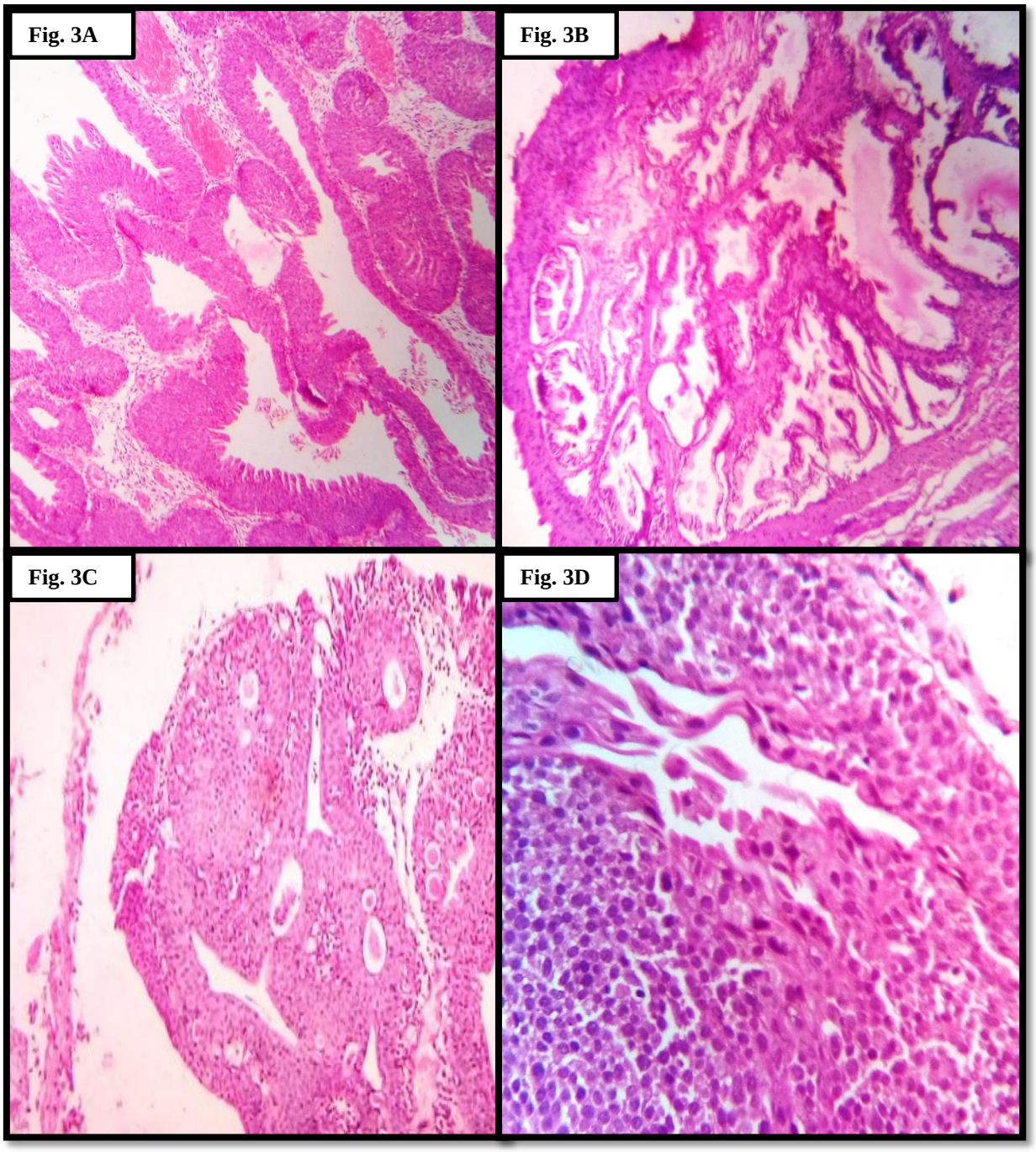

Fig-3: A) Photomicrograph showing urothelial carcinoma, Papillary type (low magnification, 10X); B) Photomicrograph showing urothelial carcinoma, with Glandular differentiation (low magnification, 10X); C) Photomicrograph showing urothelial carcinoma, Microcystic Variant (low magnification, 10X); D) Photomicrograph showing urothelial carcinoma, with Squamous Metaplasia (high magnification, 40X)

In the present study, out of 100 cases 90% tumours had papillary architecture (Fig 3A). Glandular differentiation was noted in 1% case (Fig 3B), microcyst formation in 4% cases(Fig 3C) and Squamous metaplasia was present in 5% cases (Fig 3D). In the study by Sasikumar *et al* [18], 7.4% had squamous mataplasia, and 1 out of 148 patients had glandular and Micropapillary variety each.

In this study the expression of CK20 was studied. CK20 is a marker of neoplastic change and it also points the progression to urothelial carcinoma [19-21].

It is also expressed in the metastatic lymph node from urothelial carcinoma of bladder [22]. Out of

100 cases, 13% showed 1+, 67% showed 2+, and 20% showed 3+ staining for CK20. A stage wise comparison was done for CK20 expression. It was found that in the present study among the 23 PTa cases, 8 were 1+ (34.78%), 15 were 2+ (65.22%) and none were 3+. In the study in Egypt by Raheem *et al* [23], amongst 25 cases of PTa, 1 was weakly positive (4%), 7 were moderately positive (28%), and 11 were strongly positive (44%).

Amongst the 48 cases of PT1 in the present study, 3 were 1+ (6.25%), 41 were 2+ (85.42%), and 4 were 3+ (8.33%) for CK20 as compared to 11 of 1+ (15.94%), 39 of 2+ (56.52%) and 6 of 3+ (8.70%) out of 69 PT1 cases in study by Raheem *et al* [23].

Amongst the 29 cases of PT2 in the present study, 2 were 1+ (6.90%), 11 were 2+ (37.93%) and 16 were 3+ (55.17%) as compared to 62 PT2 cases in study by Raheem *et al*, out of which 7 were 1+ (11.29%), 25 were 2+ (40.32%) and 21 were 3+ (14%) for CK20 [23].

A comparison of CK20 with invasiveness showed that in the present study, out of all the 75 invasive cases, 50 cases had 2+ staining (66.67%), 5 had 1+ (6.67%), and 20 had 3+ staining (26.67%) for CK20, compared to the 25 noninvasive cases out of which 8 showed 1+ staining (32%) and 17 showed 2+ staining (68%), while none of the cases showed 3+ staining.

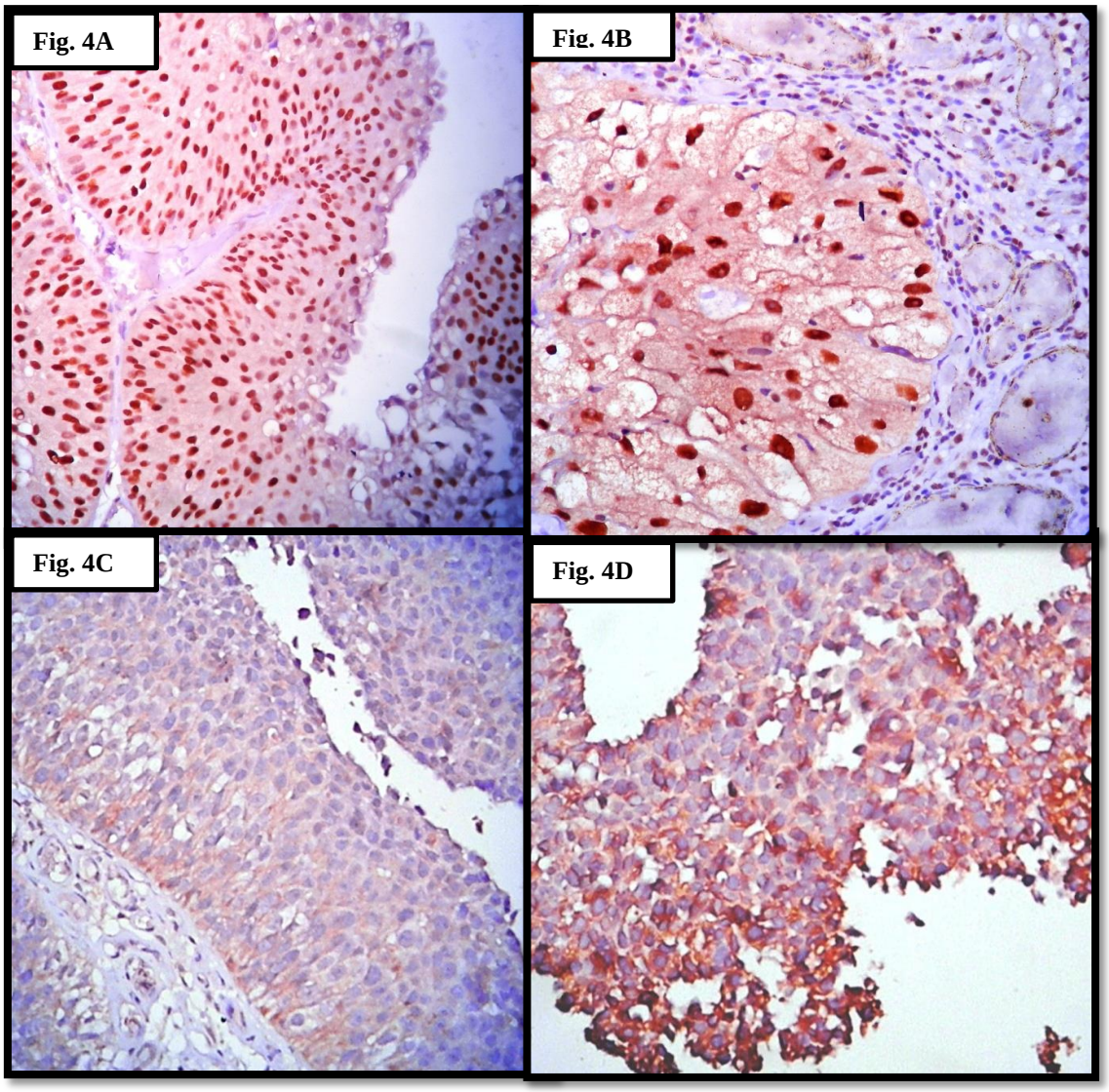

Fig-4: A) Photomicrograph showing low grade urothelial carcinoma with 2+ nuclear p63 stain (low magnification, 10X); B) Photomicrograph showing high grade urothelial carcinoma with 3+ nuclear p63 stain (high magnification, 40X); C) Photomicrograph showing low grade urothelial carcinoma with 2+ cytoplasmic CK20 stain (low magnification, 10X); D) Photomicrograph showing high grade urothelial carcinoma with 3+ cytoplasmic CK20 stain (low magnification, 10X)

A comparison of CK20 with grade showed 24.49% with 3+ staining (Fig 4B), 67.35% with 2+ staining and 8.16% with 1+ staining in High grade tumours, compared to 15.69% of 3+, 66.67% of 2+ (Fig 4A) and 17.65% of 1+ staining in Low grade tumours.

In this study among the 23 cases of PTa, 7 were 1+ (30.43%), 10 were 2+ (43.48%) and 6 were 3+ (26.09%) compared to the 25 cases of PTa of Raheem *et al* where 24 were 3+(96%) staining for P63 [23].

Out of the 48 cases of PT1 in the present study, 23 cases (47.92%) stained 2+ for P63, and 25 (52.08%) stained 3+ for p63 compared to 11 cases(15.94%), 30 (43.48%), and 25 (36.23%) of 1+, 2+, and 3+ staining of p63 respectively in the study by Raheem *et al* [23].

And out of 29 cases of PT3, 16 (55.17%) were 2+ and 13 (44.83%) stained 3+ for p63. While in study by Raheem et al out of 62 cases 18 (29.03%) stained 1+, 28 (45.16%) were 2+ and 3 (4.84%) stained 3+ for p63 [23].

A comparison of p63 staining and grade was done which showed amongst the 49 High grade cases, 2 cases stained 1+ (4.08%), 25 cases for 2+ (51.02%), 22 cases for 3+ (44.9%) (Fig 4B), compared to 51 cases of Low Grade, out of which 5 cases showed 1+ (9.8%), 24 cases showed 2+ (47.06%) (Fig 4A), and 22 cases showed 3+ (43.14%) staining for p63.

Thus in the present study, a higher CK20 and p63 staining were seen with increase in stage and grade of the tumour and was found to be statistically significant ($p<0.001$).

A higher percentage of p63 in the invasive group was noted in the present study, which may be attributed to the difference in the semiquantitative scoring used in the present as well as other studies [23].

CK20 is an epithelial marker of molecular weight 46kDa. In non-neoplastic urinary bladder epithelium, CK20 has been found to positive in surface umbrella cells only [24]. In Carcinoma-in-situ, CK20 is positive in deep urothelial cells, while in papillary urothelial neoplasms CK20 is associated with increasing tumour grade and stage as is seen in study by Desai *et al* [25]. CK20 is also positive in voided urine samples and can be detected by RT-PCR [26].

Most studies have shown CK20 immunoexpression to be associated with extent of disease as shown by Yildiz *et al*, where CK20 was found to be positive in 90% of dysplasia, 89% of CIS, and 71% of Invasive Carcinoma cases [27]. Therefore CK20 is important in cases where diagnostic difficulty arises in understanding the morphology.

P63 is a member of the p53 gene family, located at Chromosome 3q27-29. It is a myoepithelial marker, others being smooth muscle myosin heavy chain, calponin, p75, P-cadherin, basal cytokeratin (CK5/6) and CD 10 [28].

P63 is valuable in differentiating prostatic adenocarcinoma (p63 negative) from urothelial carcinoma (p63 positive) as seen in studies by Chuang *et al* and Srinivasan and Parwani [29, 30] however, as shown by Wang *et al* p63 is also negative in partial atrophy of prostate, which may be a strong mimicker [31]. P63 is also helpful in differentiating upper urinary tract urothelial carcinoma from renal cell carcinoma as seen in study by Langner *et al* [32].

CONCLUSION

Among the urinary bladder cancers, urothelial carcinoma is the most common type of neoplasm. This study was done with an aim to estimate the epidemiology of urothelial cancers and to assess the relation between various data like smoking, gender, symptoms, invasion, grade, stage. It was also done to compare the immune positivity with histomorphological and clinical patterns.

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