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Original Article

Correlation of CD24 expression with Histological Grades of Papillary Urothelial Carcinoma

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ABSTRACT

Background: Although the ratio of urinary bladder carcinoma is lowest in female than male, but in earlier sex it's grow in higher violent form of lesion, usually present at higher complex stage and poor outcome than later sex. The commonest histopathological sub-type of urinary bladder neoplasm is urothelial carcinoma. According to resent guidelines of European Association of Urology and Japanese Urology Association urothelial carcinoma of any variants are measured as high grade still if no muscular invasion.

Objective: To observe the correlation between CD-24 expressions with various histological grades of Papillary Urothelial Carcinoma at Tertiary Care Hospital Karachi

Material and Methods: This cross sectional study was conducted in the department of Pathology Basic Medical Sciences Institute (BMSI), JPMC Karachi. Amongst 83 selected cases of papillary urothelial carcinoma was reviewed and morphological diagnosis done on Hematoxylin and Eosin (H&E). Histological grading were categorized and CD-24 immuno staining done. The data analyzed by SPSS version 21.

Results: Out of 83 cases 38.5%, 37.5% and 24% cases showed invasive, high and low gradePUC respectively. In male gender 39%, 35% and 26% cases were noted in high, invasive and low grade, while amongst female 48%, 33% and19% cases were in invasive, high and low grade respectively. Most cases showing high grade and invasive tumors were noted in 5th and 6th decades. CD-24 immuno staining positivity was seen in majority of high grade and invasive PUC. In high grade 68% showed moderate and 13% showed strong immuno staining. In invasive 53% showed moderate, 31% strong positivity. Whereas 45% cases in low grade were immuno negative.

Conclusion: Majority of low grade cases of PUC were negative for CD-24 immuno staining, while 81% in high grade and 84% in invasive variant showed moderate to strong immuno staining. The intensity of CD-24 expression increased with the higher histological grades of Papillary Urothelial Carcinoma, which indicating that the CD-24 is a marker of poor prognosis.

Key words; Cluster of Differentiations (CD), Immunohistochemistry (IHC), Papillary Urothelial Carcinoma (PUC), Transurethral Resection of Urinary Bladder Tumor (TURBT)

INTRODUCTION

Malignancy is a serious health problem including urinary bladder neoplasm and becomes the leading cause of mortality in Asian Sub continent [1,2].
Globally urinary bladder cancer is the commonest neoplasm accounts for 6.5% of all malignancy and 9th to12th leading cause of death in human neoplasm [3]. According to urologist it is the 2nd commonest malignancy [4,5].
Although the ratio of urinary bladder carcinoma is lowest in female than male, but in earlier sex its grow in higher violent form of lesion and usually present at higher complex stage than their later sex counterparts and female have poor outcome[6]. The commonest histopathological sub-type of urinary bladder neoplasm is urothelial carcinoma. According to resent guidelines of European Association of Urology and Japanese Urology Association urothelial carcinoma of any variants are measured as high grade still if no muscular invasion[7].

Approximately 72,570 patients of both genders were diagnosed and 15,210 patients died in 2013 due to urinary bladder cancer. According to American Cancer Society 2006 data showed 61,420 patients were diagnosed and 13, 060 patients died due to urinary bladder malignancy[8.9].
More than 90% of urinary bladder cancer was PUC (transitional cell carcinoma), 05% was squamous cell carcinoma and 02% was adenocarcinoma [3, 10, 11].

Clinically urinary bladder cancer patients were complaints of hematuria without pain, difficulty in urination, urgency, while in advanced stage bone and flank pain with peripheral edema are the main presenting features [12-16]. Epidermal growth factor receptor was showed in more than 70% of invasion in muscular layer of invasive PUC with decreased survival[17].

CD-24 is small mucin like surface protein and contains 27 amino acid. Basically CD-24 was described as a specific B cell marker which expressed in initial stage of B cell development [18-20]. It is an alternative ligand for p-selectin, through which it functionally plays a role in metastasis of tumor cells [20-22]. Increase CD-24 expression shows poor outcome, worseness of the illness and decrease patient survival time[2,23].

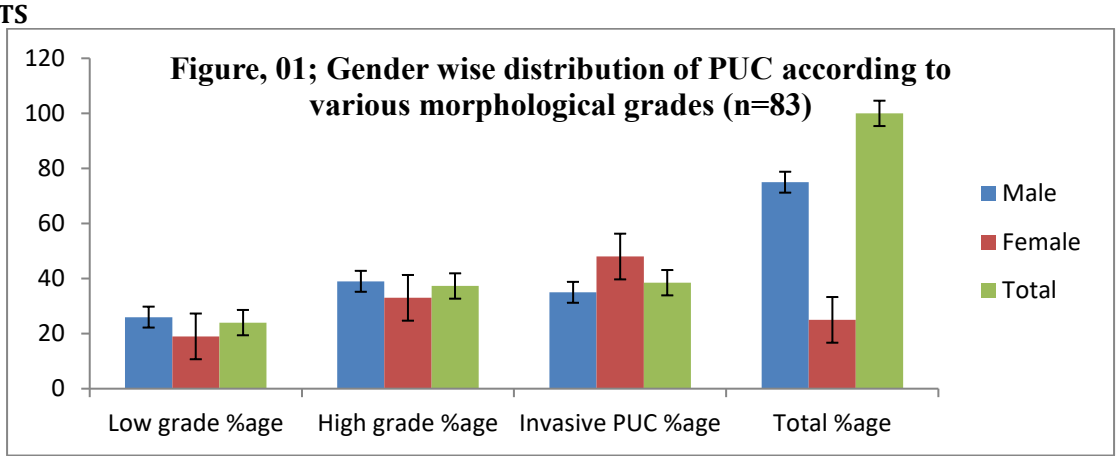
CD-24 is expressed in different solid tumor and is a prognostic marker, its expression was significantly associated with histological grades of the tumor [24]. CD-24 is novel genes in literature described as growing in malignant cells and have minimal expression in normal epithelium [25].

METERIAL AND METHODS

The study was performed at the department of Pathology BMSI, Jinnah Postgraduate Medical Center (JPMC) Karachi. Amongst received 247 cases of papillary urothelial carcinoma, after calculating sample size, 83 cases were randomly selected in this study. Moreover the procured kits allowed a limited number of cases to be stained. These patients were operated at Urology department of JPMC Karachi. All specimens in the form of transurethral resection of urinary bladder tumor and cystectomy were included, whereas poorly fixed, inadequate tissue, bladder tumor other than PUC and metastatic tumors were excluded.
Goat CD-24 (c-20); sc-7034, polyclonal antibody and Goat anti horse, sc-2448 (secondary kit) both were in concentrated form, earliest were diluted 1:25, latter were 1:200 with Phosphate Buffered Saline, they were from SANTA CRUZ BIOTECNOLOGY and horse radish peroxidase were used. Formalin fixed, paraffin embedded blocks, surgical pathology, clinical records, Hematoxylin and Eosin (H&E) slides and thin slices of five microns were made, section were cut on to poly-L-lysine coated slides for used of antibody. Slides were revived and graded on histology assigned to each case.
CD-24 expression was observed, the intensity and extent of staining each graded on scale of 1to 3.
Extent of cell was as follows;
0 = 100% cells were negative,
1+ = less than 10% cells were positive
2+ = 10-50% cells were positive and
3+ = more than 50% cells were positive.
Intensity of reactivity was as follows;
0 = no staining,
1+ = weak,
2+ = moderate and
3+ = strong immuno staining.
Intensity and extent in each case were added and additive score were categorizedas [26].
Category I = 00 to 02 score (immuno negative)
Category II = 03 to 04 score (immuno positive)
Category III = 05 to 06 score (immuno positive)

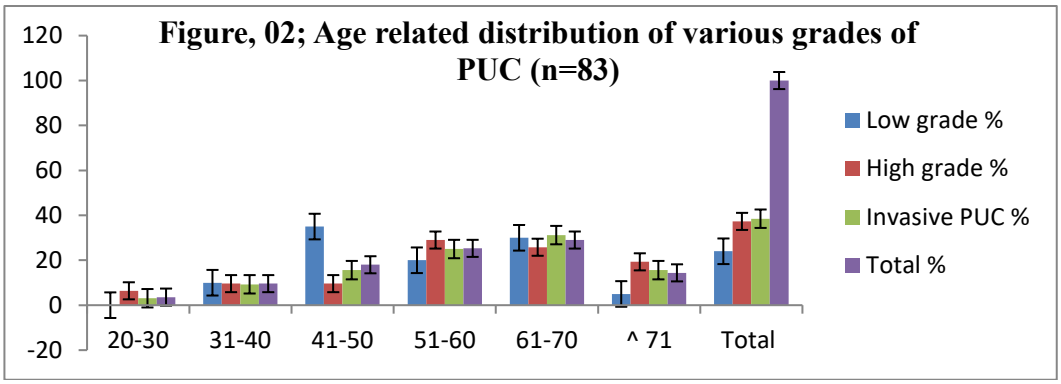
Human spleen known to be positive for CD-24 was used as positive control. The relevant clinical information and other data were collected. All slides were studied under light microscope using scanner (04X), low power (10X) followed by high power (40X) with the help of two different histopathologist. The data was analyzed by using Statistical Package for Social Sciences (SPSS) version 21 and Excel 2007.

RESULTS



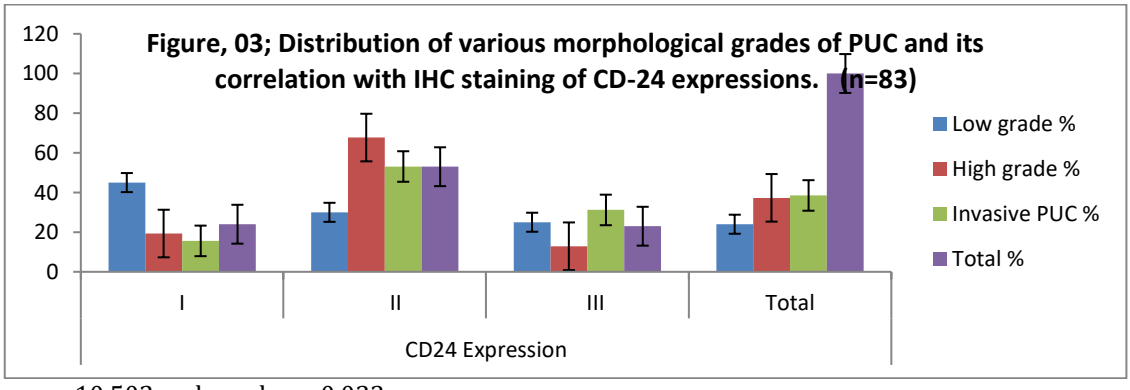
Chi-Square = 1.839 and p-value = 0.339

Figure 01; demonstrate the gender wise distribution of PUC according to various morphological grades with significant p-value. In male sex majority i-e 39% high grade 35% invasive and 26% cases in low grade. While in female sex 48% case were noted in invasive variant, followed by 33% high and 19% in low grade malignancy. However distributions of various morphological grades of PUC were 24% low, 37% high grade while 38.5% cases showed invasive variant of urothelial carcinoma.



Chi-Square = 8.273 and p-value = 0.602

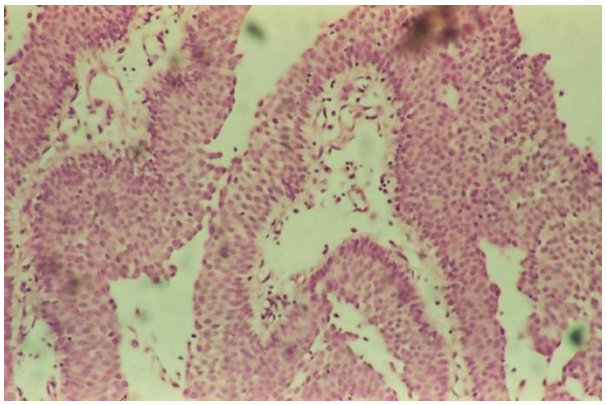
Figure, 02; showed different age groups distribution of PUC in various morphological grades. In low grade majority of cases 35% were found in 4th decades, followed by 30% in 6th decades. In high grade major fraction i- e 29% were seen in 5th decades followed by 26% cases were in 6th decades. While in invasive urothelial carcinoma most of them i-e 31% were in 6th decades followed by 25% cases were observed in 5th decades of life. The median and mean age was 58.61 and 52.5 years respectively. The minimum, maximum age was 22, 90 years and standard deviation (S-D) ± 14.24 was noted.



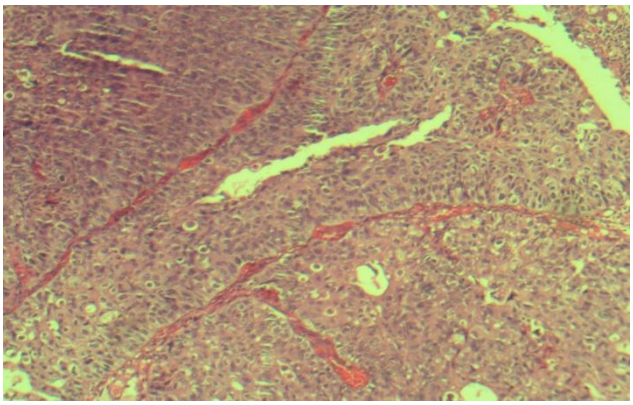
Chi-Square = 10.502 and p-value = 0.033

Figure, 03; showed the distribution of CD-24 expression in various morphological grades. Out of 20 cases in low grade majority i-e 45% showing weak staining that was considered as immuno negative (I), 30% were moderate immuno staining (II), whereas only 25% showed intense immuno positivity (III). Most of high grade cases i-e 68% showed moderate staining (II) followed by 19% of weak immuno staining (I). While in invasive urothelial carcinoma mostly cases i-e 53% were showed moderate (II) followed by 31% cases of strong immuno staining (III).

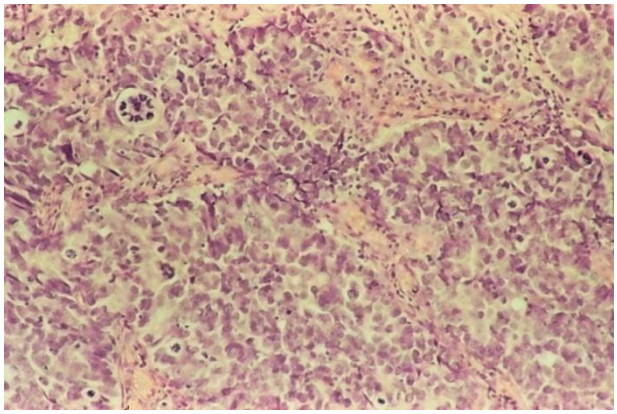
Thus our observations proved that the intensity of CD-24 expression was increased with the severity of morphological grade of PUC with significant p-value.



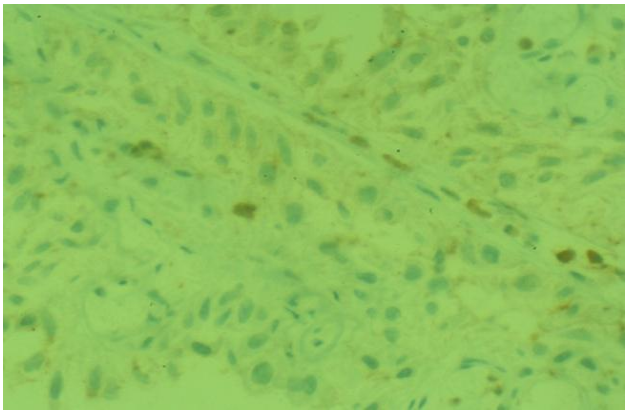
Figure, 04; High Grade PUC H&E stain (40X).



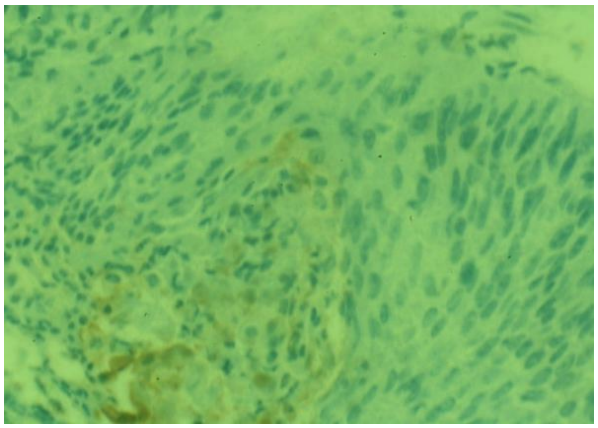
Figure, 05: Invasive PUC H&E stain (10X).



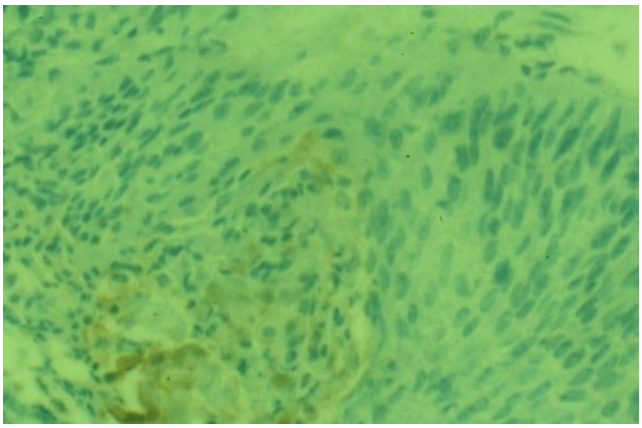
Figure, 06: Invasive PUC H&E stain (10X).



Figure, 07; Low Grade PUC with CD2-4 expressions, 2+1=II



Fig, 08; Invasive PUC with CD-24 expressions, 2+2=II.



Figure, 09; Invasive PUC CD-24 expressions, 2+2=II

DISCUSSION

The aim of this study was to detect CD-24 expression in various histological grades of papillary urothelial carcinoma and correlate with age and gender.

Our study showed that maximum number 31%, 26% cases of PUC of invasive and high grade were presented in 6th decades' of life, while 35% of low grade were observed in 4th decades'. These figures were comparable with Iranian study by Salehi *et al* (2011) [4] who reported 40% cases of urothelial carcinoma were seen in 70 or above age groups. Indian study by Biswas*et al* (2013) [27] reported 35.2% cases in 6th decades', followed by 27% in 5th decades' of life. Chinese study by Zhang *et al*(2012) [28] reported that mostly cases of urothelial carcinoma were seen between 5th to 7th decades. According to American Cancer Society by Tripathi *et al* (2002) [29] reported that relatively risks for urothelial carcinoma were increased from 1.00 in 55 years to 1.65 rates in above 65 years of age.

Our observations showed that low grade was more i-e 65% in 4th, 3rd and 5th decades, high grade were 74 % in 5th, 6th, 7th and above age and invasive PUC were 72% in 6th, 5th, 7th and above age. Low grades more in 4th decades i-e 35%, high grade in 5th decades i-e 29% and invasive in 6th decades i-e 31% respectively. Comparable with study by Biswas *et al* (2013) [27] reported 27.2% in 5th and 35.2% in 6th decades. Zhang *et al* (2012) [28] showed that majority of PUC i-e 70% to 72% cases were seen in above 55 years of age groups.

In present study aimed to detecting CD-24 expression in various grades of papillary urothelial carcinoma were noted as 45% cases of low grade were immuno negative, however considerable number i-e 30% and 25% cases of low grade tumor showed moderate to high immuno reactivity with CD-24 expression. It can be assumed that these cases probably represent a population that would eventually show early recurrence and high progressive course. Majority of high grade tumor i-e 68% and 12.9% cases show moderate to high immuno staining. Most likely these cases would show an aggressive behavior. The high grade cases showing 19.3% immuno negativity may be assumed to take less aggressive course. Whereas 53% invasive urothelial carcinoma showed moderate and 31% cases showed strong immuno staining. These figures were comparable with Japanese study by Liu *et al* (2013) [30] who reported 72.4 % of low grade were immuno negative and 74% cases in high grade were immuno positive. Korean study by Choi *et al* (2007) [18] showed 52% cases in low grade were immuno negative and 77.8% cases of high grade were moderate immuno staining. None of them reported any case as invasive variant while our data showed 38.5% cases in this variant with 84% of moderate to strong immuno staining and our result showed significant association between CD-24 expression and urothelial carcinoma particularly in invasive carcinoma.

CD-24 expressions have strong association with the aggressiveness' of the disease. Whenever its extent and intensity were high than it may have worse prognosis, Liu *et al* (2013) [30] reported that recurrence of the bladder tumor case in low grade i-e 27.6% and in high grade i-e 74% with high mortality rate. Choi *et al* (2007) [18] also reported that CD-24 expression was significantly higher in invasive urothelial carcinoma than in non invasive. Due to high recurrence of bladder cancer CD-24 has a prognostic significance and proven as prognostic marker.

CONCLUSION AND RECOMMONDATION

- CD-24 expression progressively increased with higher histological grades, the intensity of CD-24 staining was directly proportional to the severity and grades of PUC which showed that significant correlation was found between histological grades and CD-24 expression, thus proving that CD-24 is a marker of poor prognosis.
- The use of immuno histochemistry to evaluate protein levels is only semi-quantitative, therefore molecular studies along with CD-24 immuno histochemistry and molecular PCR are recommend to identifying genetic mutation, and long term follow up studies are recommended to observe the course of such tumors.

CONFLICT OF INTEREST; The author's declare no financial or non-financial competing interests**.**

AUTHOR'S CONTRIBUTION

- **ISM, GS & LY →** conceived/design, analysis and editing of manuscript.
- **RF, KSM & PAJ →** helps in data collection, drafting the work & manuscript and statistical analysis
- **ISM, GS, RN & SA →**did review final approval of manuscript and agreement to be accountable.

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