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# CLINICOPATHOLOGICAL SIGNIFICANCE OF OCT4 EXPRESSION IN PATIENTS WITH CARCINOMA OF THE URINARY BLADDER

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## INTRODUCTION

Bladder cancer ranks as the most common neoplasm involving the urinary tract and the ninth most prevalent malignant tumor in the world.[1] It is the sixth most prevalent malignant tumor in Iraq.[2] The most common type of bladder tumors diagnosed is transitional (urothelial) cell carcinoma (TCC); it constitutes more than 90% of bladder cancers.[3] Urothelial carcinoma is a highly heterogeneous disease that develops along distinct.

Biological tracks Recently identified a subset of cells known as urothelial cancer stem cells (UroCSCs) in urothelial cell carcinoma (UCC) have self-renewal properties, ability to generate cellular tumor heterogeneity via differentiation and Are ultimately responsible for tumor growth and viability.[4]

The therapeutic resistance of cancer stem cells (CSCs) to commonly used cancer therapies, including chemotherapy and radiotherapy, is considered a major obstacle in the treatment of different cancers. Post-therapeutic recurrence commonly occurs in BC,[4].

Characteristic markers and proteins may help to identify bladder CSCs and thus early stages of bladder cancer. OCT-4 (the gatekeeper of self-renewal), also known as POU domain, class 5, transcription factor 1 (POU5F1), is a protein that in humans is encoded by the POU5F1 gene.[5] OCT-4 is a homeodomain transcription factor of the POU family. It is critically involved in the self-renewal of undifferentiated embryonic stem cells. As such, it is frequently used as a marker for undifferentiated cells. OCT-4 is a member of the octamer transcription factor family, so named because they bind the octameric (8-unit) DNA nucleotide sequence ATTTGCAT.[6] The OCT-4 transcription factor is initially active as a maternal factor in the oocyte and remains active in embryos throughout the pre-implantation period. OCT-4 can form a heterodimer with SOX2 so that these two proteins bind DNA together.[7] Mouse embryos that are OCT-4 deficient or have low expression levels of OCT-4 fail to form the inner cell mass, lose pluripotency, and differentiate into trophectoderm.[8] Therefore, the level of OCT-4 expression in mice is vital for regulating pluripotency and early cell differentiation since one of its main functions is to keep the embryo from differentiating.[9] In a mature organism, OCT4 is not present in mature and differentiated cells and is found only in germ cells. OCT4 gene encodes three transcripts and four protein isoforms that are generated by alternative splicing, OCT4A, and OCT4B, and OCT4B1. It is suggested that OCT4A and OCT4B can be distinguished by their distinct subcellular localization.[10] Only the OCT4A form, which is present in cell nuclei, exhibits transcription factor functions and is responsible for maintaining cells at an undifferentiated stage, stem cell properties, and the ability for self-renewal. OCT4 regulates the expression of several target genes, including NANOG, SOX-2, REX-1, and CDX-2, involved in the regulation of pluripotency. OCT4 is generally considered a universal marker of pluripotent stem cells.[11] OCT4 expression in cancer cells: The presence of OCT4 protein is associated with, for example, poor prognosis in non-small-cell lung cancer, hepatic cancer, and esophageal squamous cell carcinoma. One possible mechanism responsible for the more aggressive behavior of cancers and worse clinical outcomes with cells expressing OCT4 is the presence of the stem cell phenotype in cancers related to OCT4-mediated dedifferentiation and related chemoresistance.[12]

## METHODS

A total number of 60 tissue samples were collected for the study, 30 samples of Group A retrospective obtained from archives of histopathology unit with 30 prospective samples were obtained from the Directorate of Forensic Medicine. The patients' medical reports, with full histopathological parameters, werecollected and reviewed. After appropriate trimming, a serial of four micrometer-thick tissue sections was obtained using the automated microtome. For each case, two sections were taken; the first was placed on an ordinary slide and stained with hematoxylin and eosin to confirm the diagnosis and to determine the histological type and

grade for the tumor and the second section was put on the positively charged slides for immunohistochemical staining with anti-OCT4 antibody. Immunohistochemical staining Slides preparation was placed in semi-vertical position in the oven at 65°C overnight. The slides were covered by water until ready to perform antigen retrieval; they should be kept wet because it will yield a non-specific antibody binding. • Heat-induced epitope: Slides were put in a vertical position then put in 250 ml (10 mmol sodium citrate buffer complete with wash buffer, ph 6) in a plastic container then cover and heated at 95 for 5 min allow the slides to cool in the buffer for approximately 20 min. Wash in deionized H2 O 3 times for 2 min each, aspirate excess liquid from slides. • Peroxidase block: Incubate for 7–10 min in 50 ul hydrogen peroxide in a humid chamber to quench endogenous peroxidase activity. Wash in phosphate-buffered saline (PBS) twice for 5–7 min each then drained. • Protein block: The slides were incubated with protein block UltraCruz® blocking reagent in a humid chamber for 1 h to eliminate non-specific background staining then drained for a few seconds without a rinse and wipe around with a piece of tissue paper. • Primary antibody: 50 ul of prediluted primary antibody was placed into sections (dilution 1:70 for OCT4) incubated in a wet chamber at 4°C overnight for SOX2. • The slides were washed with fresh PBS twice for few minutes each. Then, the slides were drained. • Conjugated secondary antibody enough drops of secondary antibody were applied to cover the specimen and incubated in a humid chamber at room temperature for 60 min. Then, the slides rinse with PBS 2 × 5 min then drained and blotted. • Substrate chromogen solution: 50 drops of diaminobenzidine (DAB) substrate with one drop of chromogen were mixed, few drops were added and incubated for 10 min in the humid chamber or until desired stain intensity develops, then washed with tap water for few minutes each. • Counterstain with Mayer's hematoxylin was used for 1 min, then washed with tap water, followed by distal water for few minutes then slides were drained and blotted. • Mounting: One to two drops of mounting media are applied onto the sections, then covered with coverslips and left to dry overnight. Evaluation of immunostaining scores The cells were scored as positive or negative staining depending on the presence of distinct brown nuclear staining. The accuracy of the positive and strongly positive categories was further tested and confirmed by ranking each slide from the lowest to highest intensity and extent of staining and location was also revealed for each marker. The slides were examined with low-power microscopy ×10 to determine the regions of highest staining, if they show no staining at low power, reexamination was done by high power ×400 to determine area of weak staining, five fields of each slide were examined and scored semi-quantitatively by calculating the proportion of positively stained cells over the total number of tumor cells examined (%) and samples were graded according to the extent of staining and intensity. Statistical analysis The data analyzed using the Statistical Package for the Social Sciences version 25. The data presented as mean, standard deviation, and ranges. Categorical data presented by frequencies and percentages. Pearson's Chi-square test was used to assess the statistical association between different associated variables.

## RESULTS

Current Study patient's age was ranging from 40 to 84 years with a mean standard deviation (SD) of 69.52 ± 11.17 years. Male predominance noticed (83.3% versus 16.7%, with male: female ratio 4:1.

There was association between the OCT4 marker result and certain clinicopathological features is shown in Table 2. The highest prevalence of positive OCT4 result was found in patients with high grade tumor (90.9%) with a significant association (p=0.013) between OCT4 result and the grade. Regarding muscular invasion, we noticed that 87.5% of patients with muscular invasion showed positive OCT4 marker result with a significant association (p=0.039) between OCT4 marker result and muscular invasion. There was no significant association (p≥0.05) between OCT4 marker result and other clinicopathological features of patients (Fig. 1).

| Variable | OCT4 Result | | Total n= 60 | P – value |
|---|---|---|---|---|
| | Positive (%) n= 48 | Negative (%) n= 12 | | |
| **Age (Years)** | | | | |
| **51 – 60** | 10 (71.4) | 4 (28.6) | 7 (23.3) | 0.809 |
| **61 – 70** | 28(82.4) | 6 (17.6) | 17 (56.7) | |
| **71 – 80** | 10 (83.3) | 2 (16.7) | 6 (20.0) | |
| **Gender** | | | | |
| **Male** | 42(84.0) | 8 (16.0) | 25 (83.3) | 0.22 |
| **Female** | 6 (60.0) | 4(40.0) | 5 (16.7) | |
| **Grade of the tumor** | | | | |
| **High** | 40 (90.9) | 4 (9.1) | 22 (73.3) | 0.013 |
| **Low** | 8 (50.0) | 8 (50.0) | 8 (26.7) | |
| **Lamina properia invasion** | | | | |
| **PRESENT** | 20 (71.4) | 8(28.6) | 14 (46.7) | 0.272 |
| **ABSENT** | 28 (87.5) | 4(12.5) | 16 (53.3) | |

| | | | | |
|---|---|---|---|---|
| **Muscularis properia invasion** | | | | |
| **PRESENT** | 42 (87.5) | 6 (12.5) | 24 (80.0) | 0.039 |
| **ABSENT** | 6 (50.0) | 6(50.0) | 6 (20.0) | |
| **squamous metaplasia** | | | | |
| **PRESENT** | 18 (69.2) | 8 (30.8) | 13 (43.3) | 0.197 |
| **ABSENT** | 30 (88.2) | 4(11.8) | 17 (56.7) | |
| **Invasion to the adjacent organs** | | | | |
| **PRESENT** | 12 (100) | 0 (0) | 6 (20.0) | 0.17 |
| **ABSENT** | 36 (75) | 12 (25) | 24 (80.0) | |

## DISCUSSION

Current Study patient's age was ranging from 40 to 84 years with a mean standard deviation (SD)of 69.52 ± 11.17 years. Male predominance noticed (83.3% versus 16.7%, with male: female ratio 4:1.

In constituent to study conducted in Jordan (2008), as they found male predominance in their results (86% vs 14%) with a male: female ratio 9:1 and the mean age of the patients were 60.6 (range 19-91) years 7 while lower than our results in regard to gender noticed in a study conducted in China (2007) involved 49 patients with bladder carcinoma, they noticed that there were 32 males (65.3%)and 17 females (34.7%)with male: female ratio was 1.8:1 and similarly in regard to age as was ranging from 44 to 80 years old (mean 63 years).[8]

Also, agreement with an Iranian study done in 2013, in which 138 cases were male (87%) and 21 cases was female (13%) with Male: Female ratio was 6.5:1. The mean age at the time of diagnosis was 64±12 years (range 23-87 years) (Keymoosi H et al, 2014) and Egyptian one in 2016, found that 76 male cases (90.5%) and eight female cases (9.5%), with a ratio of males-to-females of 10: 1. Age ranged between 42 and 82 years, with a mean age of 61.2±9.043 years 9(Asar A et al, 2017).

Our results also showed that there was a significant association between the OCT4 positive result and inflammation with necrosis (p=0.013) in 90.9% of patients. Furthermore, with muscular invasion, it significantly related (p=0.039, 87.5%) of patients and no significant association (p≥0.05) with other clinicopathological features. Similarly, a study conducted in Japan (2011) showed that the immunohistochemical analysis demonstrated that the positive rate of OCT4 expression was significantly associated with higher grade cancer (G2 and G3) in comparison with that of the lower grade (G1).[16] Furthermore, in Iran (2017), researchers found that there was a significant correlation between the expression of OCT4 and the tumor stage.

## CONCLUSION

OCT4 can be considered as a key regulator of tumor progression, aggressive behavior, and metastasis. Furthermore, it is a reliable marker for the early diagnosis and the designed chemotherapy of bladder cancer.

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