# Comparative Study of GATA3 and CD147 Expression in Urinary Bladder Carcinoma

Nehal Abd El-Ghaffar Heabah, M.Sc., Mohammed Farid Aref, M.D, Mohamed Alaa Mokhtar, M.D, Mohamed Moustafa Shareef, M.D, Ahlam Mohamed Abo El-Enain, M.D.

Pathology Department, Faculty of Medicine, Tanta University, Egypt
nehalghaffar@yahoo.com, nehal.heaba@med.Tanta.edu.eg

**Abstract: Introduction**: Urinary bladder carcinoma is a common urologic malignancy, particularly in males. We studied GATA3 and CD147experssionin relation to tumor grade and stage, with special emphasis on the diagnostic role of GATA3 in urinary bladder carcinoma. **Materials and methods:** Paraffin blocks from 85 cases of bladder carcinoma: 69 urothelial carcinoma cases, 10 squamous cell carcinoma cases, 5 adenocarcinoma cases and one case neuroendocrine small cell carcinoma, were stained by GATA3 and CD147 immunohistochemical markers. **Results:** GATA3 exhibited high sensitivity (87%) and specificity (100%) as a diagnostic marker for urothelial carcinoma. Strong GATA3 expression had been found in all cases of low grade, plasmacytoid, microcystic, micro-papillary and clear cell urothelial carcinoma, while showed a range of sensitivity as a marker for urothelial carcinomas with variant morphologic features as squamous and sarcomatoid differentiation. A statistically significant relations were found between GATA3 expression and tumor grade (P-value=0.010) and stage (P-value=0.006). Decreased GATA3 expression was associated with high grade, muscle invasive bladder carcinoma. Regarding CD147 expression, positive CD147 staining was significantly associated with high tumor grade (P-value=0.001) and muscle invasion (P-value=0.001). There was an inverse relationship between GATA3 and CD147 (P-value=0.032). **Conclusions:** GATA3 seemed to be a valuable tool in confirming the urothelial origin of microcystic, micropapillary, plasmacytoid and clear cell urothelial carcinoma. A statistically significant relation was found between GATA3 and CD147 expression. Positive CD147 expression was associated with high grade and stage, while GATA3 positive expression was associated with low grade and stage.

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**Key words:** Urinary bladder carcinoma, GATA3, CD147.

## 1. Introduction:

Urinary bladder carcinoma (UBC) is an international public health problem **(Malats and Real, 2015)**. It is the 9th frequently-diagnosed cancer worldwide, the 7th common cancer in men and the 14th leading cause of deaths due to cancer worldwide **(Mahdavifar et al., 2016)**. The highest incidence of bladder cancer is observed in Europe, the United States and Egypt, respectively, while the lowest level is in Sub- Saharan Africa, Asia and South America, respectively **(Antonia et al., 2017).**

In Egypt, bladder cancer incidence and behavior differ significantly from the developed countries, as bladder cancer has been and remains one of the most prevalent malignancies, accounting for 12.22% of male cancers and representing the main bulk of the urinary system malignancy **(Salem and Mahfouz, 2012)**.

In the developed countries, approximately 70-80% of patients with newly diagnosed bladder cancer present with non- muscle invasive (MNI) or early invasive disease (stage Ta or T1). In Egypt, most UBC cases are muscle-invasive (MI) (stage T2, T3 or T4) at time of diagnosis, which may reflect the delay in seeking medical advice till late advanced disease state. This difference has made the use of particular urinary markers a focus of interest with the hope of more accurate diagnosis, grading, prediction of the biological behavior, as well as determination of their potentials as therapeutic targets in UBC patients **(Abd El-Rehim et al., 2013).**

GATA3 binding protein is a zinc-finger transcription factor which belongs to a distinct family of tumor suppressor genes that plays a critical role in the embryologic development of several tissues, including T lymphocytes, the urinary tract, and the breast **(Higgins et al., 2007)**. It is involved in human cancer cell growth and differentiation, and plays an important role in cell proliferation and apoptosis **(Mohammed et al., 2016).**

In epithelial neoplasms, GATA3 was expressed in > 90% of primary and metastatic ductal and lobular carcinomas of the breast, urothelial, and cutaneous basal cell carcinomas, trophoblastic and endodermal sinus tumors. GATA3 is highly expressed in bladder carcinoma, with a strong sensitivity as a marker of primary bladder carcinomas with purely urothelial differentiation **(Verduin et al., 2015)**. GATA3

consistently shows higher expression levels than the traditional urothelial markers: thrombomodulin and uroplakin III **(Rochester et al., 2017)**. However, the expression of GATA3 in bladder carcinomas with variant histopathological patterns has been incompletely studied **(Miettinen et al., 2014)**.

Regarding the prognostic role of GATA3, strong GATA3 expression could be considered as a predictive factor for good patient survival, as reductions in GATA3 expression correlated with invasive and high-grade of bladder cancer. There is also a relation between high GATA3 expression and good BCG response **(Nunez-Nateras et al., 2014 & Bahria Sediki et al., 2016).**

CD147 or Extracellular matrix metalloproteinase inducer, EMMPRIN (also designated basigin), is a multifunctional cell surface glycoprotein that belongs to the immunoglobulin superfamily, which is involved in the regulation of the tumor microenvironment via monocarboxylate transporters (MCTs). CD147 stimulates the production of interstitial collagenase, gelatinase A, stromelysin-1 and various metalloproteinases (MMPs) by fibroblasts, which are important factors in cancer invasion and metastasis **(Afonso et al., 2015 & Bovenzi et al., 2015)**. CD147 also stimulates tumor cell-induced angiogenesis via the stimulation of vascular endothelial growth factor production and multidrug resistance via hyaluronan-induced signaling **(Dai et al., 2013)**. It has been described that CD147 enhances tumor chemoresistance via the phosphatidylinositol 3-kinase (PI3K) pathway **(Mujtaba et al., 2017).**

CD147 expression levels can be used as an independent prognostic factor for survival. CD147 has been also defined as a potential target for tumor therapy in preclinical studies **(Hemdan et al., 2015).**

## 2. Materials and methods:

This study was carried out on 85 cases of urinary bladder carcinomas. These cases were selected from the archives of the Pathology department, Faculty of Medicine during the period from January 2017 to December 2018. Approval from the research ethics committee (REC), was taken before conducting the study. The specimens obtained were: 71 specimens of trans-urethral resection of bladder tumors (TURBT) (83.5%) and 14 radical cystectomyspecimens (16.5%).

**Histopathological study**

Histological sections, 4-mm thick, were stained by hematoxylin and eosin (H & E) for evaluation of histopathological parameters, including: the histopathological grade, depth of invasion (T), vascular, perineural invasion, associated CIS and whenever possible, lymph node status (N) and prostatic involvement.

- The studied urinary bladder carcinoma cases were classified microscopically according to the 2016 World Health Organization (WHO) classification system **(Moch et al., 2016).**
- Pathological Staging of the studied tumors was done according to American Joint Committee on Cancer (AJCC) TNM Pathologic Staging of Urinary Bladder Carcinomas **(Edge et al., 2010).**

**Immunohistochemical staining**

Immunohistochemical staining was performed using the streptavidin-biotin method as described by **(Buchwalow and Böcker, 2010)**, for evaluation of GATA3 and CD147 expression. From each paraffin block, 4-mm-thick sections mounted onto positively charged slides, deparaffinized and rehydrated. Endogenous peroxidase was blocked by immersion in 3% hydrogen peroxide. Antigen retrieval using microwave oven was performed. The primary antibodies used were:

- GATA3 antibody was mouse monoclonal antibody raised against human recombinant GATA-3, (HG3-31): sc-268 (**Santa Cruz Biotechnology, Inc**). Two to three drops of GATA3 were placed on each slide at a 1:100 dilution.
- CD147 antibody was a mouse monoclonal antibody raised against human T-cell leukemic cell line, Peer, (8D6): sc-21746 (**Santa Cruz Biotechnology, Inc**). Two to three drops of CD147 were placed on each slide at a 1:100 dilution.

**Interpretation of GATA3 positivity:**

- Positive staining was indicated by the presence of brownish nuclear staining in the tumor cells of the studied cases.
- **Miyamoto et al., 2012**, used the German immunoreactivescore, that "multiplies" both the percentage of immunoreactive cells and the staining intensity.

➢ **The percentage of immunoreactive cells**:0% (0), 1%-10% (+1), 11%-50% (+2), 51%-80% (+3), 81%-100% (+4).

➢ **The staining intensity was scored as:** Negative (0), Weak (+1), Moderate (+2), Strong (+3).

- **GATA3 IHC scoring results ranged from (0-12):**
  - ✓ From (0-1): Negative (0).
  - ✓ From (2-4): Weakly positive (+1).
  - ✓ From (6-8): Moderately positive (+2).
  - ✓ From (9-12): Strongly positive (+3).

**Interpretation of CD147 positivity:**

- CD147 staining was detected as brownish membranous staining in the tumor cells of the studied cases.
- **Afonso et al., 2015**, used semiquantitative grading system, considering the “sum” of the

percentage of immunoreactive cells and the staining intensity.

➢ **The percentage of immunoreactive cells**:0% positive cells (0), <5% of positive cells (+1), 5–50% positive cells (+2), >50% of positive cells (+3).

➢ **The staining intensity was scored as:** Negative (0), Weak (+1), Moderate (+2), Strong (+3).

- **CD147 IHC scoring results ranged from (0-6):** final **scores ≥4** were considered positive.

**Statistical analysis**

The collected data was statistically analyzed using SPSS software statistical computer package version 20. Data were expressed in terms of frequencies (number of cases) and percentages for categorical variables and range, median, mean±standard deviation (SD) for continuous variables. For comparing categorical data, Chi-square (X2) test was used as a test of significance. Fisher's exact test or Monte Carlo was used when one or more of cells have an expected frequency of five or less. P values of <0.05 were considered statistically significant **(Petrie and Sabin, 2005).**

➢ **For confirmation of the diagnostic role of GATA3 in urothelial carcinoma, the following formulas were used:**

- **Senstivity (True positive results):** True positive/(True positive + False negative) × 100.
- **Specificity (True negative results):** True negative/(True negative + False positive) × 100.
- **Positive predictive value (PPV):** True positive/(True positive + False positive**)** × 100.
- **Negative predictive value (NPV):** True negative/(True negative + False negative) × 100.
- **Accuracy:** (True positive + True negative) / (True positive + True negative + False positive + False negative) × 100.

## 3. Results:

**Histopathological examination of the studied cases:**

The 85 studied cases (78 cases were males, 91.8% and 7 cases were females, 8.2% with age ranged between 38 and 86 years, mean age was 61.08), were categorized into 4 groups:

(I) Group I: 69 cases of urothelial carcinoma (UC) (81.2%): including 10 cases of non-invasive low grade papillary UC and 59 cases of infiltrating UC. Infiltrating urothelial carcinomas included: 29 case pure UC, 13 cases UC with squamous differentiation, 4 cases UC with glandular differentiation, 3 cases sarcomatoid UC, 2 cases plasmacytoid UC, 2 cases microcystic UC, 2 cases micropapillary UC, 2 cases clear cell UC, one case UC with both squamous and glandular differentiation, and one case poorly differentiated urothelial carcinoma.

- UC cases were graded into: 18 cases low grade urothelial carcinoma (26%) and 51 cases high grade urothelial carcinoma (74%).
- Regarding muscularispropria (MP) invasion in urothelial carcinoma group: 23 cases were NMI "Ta & T1" (33.3%) and 46 cases were MI "T2, T3 & T4" (66.7%).

(II) Group II: 10 cases of squamous cell carcinoma (SCC) (11.8%).

Eight cases were moderately differentiated SCC and two cases were poorly differentiated SCC.

(III) Group III: 5 cases of moderately differentiated adenocarcinoma (5.9%).

(IV) Group IV: One case of neuroendocrine small cell carcinoma (SmCC) (1.1%), comfirmed by strong membranous immunoreactivity to CD56 neuroendocrine marker.

**GATA3 immunohistochemical results**

The relation between GATA3 expression and the studied groups was statistically significant **(P-value= 0.001)**, as 60 cases of urothelial carcinoma were GATA3 positive (87%), while all cases of SCC, adenocarcinoma and neuroendocrine small cell carcinoma were GATA3 negative (Table 1)**.**

- Strong nuclear GATA3 expression (+3) was noted in all cases of: Low grade non-invasive papillary UC, plasmacytoid, microcystic, micropapillary and clear cell UCs (Figure 1).

Regarding the remaining histopathological variants of UC, GATA3 showed a range of sensitivity as follows:

- Infiltrating pure UC: 12 cases showed strong nuclear positivity for GATA3 (+3) (66.7%), 4 cases showed moderate GATA3 expression (+2) (22.2%) and 2 cases were GATA3 negative (11.1%).
- UC with squamous differentiation: the urothelial component of 10 cases showed strong positive GATA3 expression and 3 cases were GATA3 negative, while its expression in the squamous component was variable, as 2 cases showed strong GATA3 positivity (+3) (15.4%)**,** 5 cases were moderately positive (+2) (38.5%)**,** 3 cases showed weak GATA3 positivity (+1) (23.1 %) and 3 cases were GATA3 negative (23.1%) (Figure 2).
- UC with glandular differentiation: the urothelial component in these cases was strongly GATA3 positive, whereas the glandular component in 3 cases showed strong GATA3 positivity (+3) (75%) and one case was GATA3 negative (25%).
- Sarcomatoid UC: one case showed weak positivity for GATA3 (+1) in both urothelial and sarcomatoid components (33.3%) and2 cases were GATA3 negative in both urothelial and sarcomatoid components (66.7%) (Figure 3)**.**

- Lymph node metastases were present in seven of our studied cases, all showed strong positive nuclear GATA3 expression (+3) in both the primary tumor and the nodal secondary deposits.

➢ The relation between GATA3 expression and the histopathological grade in UC group was statistically significant **(P-value= 0.010)** (Table 2).

➢ The relation between GATA3 expression and muscle invasion in UC group was statistically significant **(P-value= 0.006)** (Table 3).

➢ GATA3 exhibited high sensitivity (87%) and specificity (100%) in the diagnosis of urothelial carcinoma (Table 4).

**CD147 immunohistochemical results**

The relation between CD147 expression and the four groups was statistically significant **(P-value= 0.001)**, as 66.7% of the urothelial carcinoma cases showed positive CD147 expression, all SCC cases were positive (100%), 4 cases out of 5 adenocarcinoma cases were CD147 positive (80%) and the neuroendocrine small cell carcinoma case showed also positive CD147 expression (Figure 4 and Table 5)**.**

➢ The relation between CD147 expression and grading in UC cases was statistically significant **(P-value=0.001)**. Most low grade tumors showed CD147 negative expression, while most high grade urothelial carcinomas showed positive CD147 expression (Table 6).

➢ The relation between CD147 expression and muscle invasion was statistically significant **(P-value= 0.001)**. Most of CD147 negative cases were NMI, while most of CD147 positive cases showed MP invasion (Table 7).

The relation between GATA3 and CD 147 was statistically significant **(P-value= 0.032)**, with an inverse relationship between GATA3 and CD147. CD147 positive expression is associated with higher grade and stage, while GATA3 positive expression is associated with low grade and stage (Table 8).

**Table 1: GATA3 expression among the four studied groups**

| Group | | GATA3 Scoring | | | | Total positive | Total |
|---|---|---|---|---|---|---|---|
| | | Negative | Weak +1 | Moderate +2 | Strong +3 | | |
| Urothelial Carcinoma | N | 9 | 4 | 9 | 47 | 60 | 69 |
| | % | 13% | 5.8% | 13% | 68.1% | 87% | 100% |
| Squamous cell carcinoma | N | 10 | 0 | 0 | 0 | 0 | 10 |
| | % | 100% | 0% | 0% | 0% | 0% | 100% |
| Adenocarcinoma | N | 5 | 0 | 0 | 0 | 0 | 5 |
| | % | 100% | 0% | 0% | 0% | 0% | 100% |
| Neuroendocrine carcinoma | N | 1 | 0 | 0 | 0 | 0 | 1 |
| | % | 100% | 0% | 0% | 0% | 0% | 100% |
| Total | N | 25 | 4 | 9 | 47 | 60 | 85 |
| | % | 29.4% | 4.7% | 10.6% | 55.3% | 70.6% | 100% |
| Chi-square | $X^2$ | 47.304 | | | | | |
| | P-value | MC 0.001* | | | | | |

MC: Monte Carlo for Chi square test

**Table 2: The relation between GATA3 expression and histopathological grade in UC cases**

| Grade | | GATA3 Scoring | | | | Total positive | Total |
|---|---|---|---|---|---|---|---|
| | | Negative | Weak +1 | Moderate +2 | Strong +3 | | |
| Low Grade | N | 0 | 0 | 0 | 18 | 18 | 18 |
| | % | 0% | 0% | 0% | 100% | 100% | 100% |
| High Grade | N | 9 | 4 | 9 | 29 | 42 | 51 |
| | % | 17.6% | 7.8% | 17.6% | 56.9% | 82.4% | 100% |
| Total | N | 9 | 4 | 9 | 47 | 60 | 69 |
| | % | 13% | 5.8% | 13% | 68.1% | 87% | 100% |
| Chi-square | $X^2$ | 11.399 | | | | | |
| | P-value | MC 0.010* | | | | | |

MC: Monte Carlo for Chi square test

**Table 3: The relation between GATA3 expression and muscle invasion in UC cases**

| Stage | | GATA3 Scoring | | | | Total positive | Total |
|---|---|---|---|---|---|---|---|
| | | Negative | Weak +1 | Moderate +2 | Strong +3 | | |
| Non-muscle invasive | N | 1 | 0 | 0 | 22 | 22 | 23 |
| | % | 4.3% | 0% | 0% | 95.7% | 95.7% | 100% |
| Muscle-invasive | N | 8 | 4 | 9 | 25 | 38 | 46 |
| | % | 17.4% | 8.7% | 19.6% | 54.3% | 82.6% | 100% |
| Total | N | 9 | 4 | 9 | 47 | 60 | 69 |
| | % | 13% | 5.8% | 13% | 68.1% | 87% | 100% |
| Chi-square | $X^2$ | 12.340 | | | | | |
| | P-value | MC 0.006* | | | | | |

MC: Monte Carlo for Chi square test

**Table 4: Sensitivity and specificity of GATA3 in the diagnosis of urothelial carcinoma**

| GATA3 Scoring | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|
| Urothelial carcinoma | 87% | 100% | 100% | 64% | 89% |

**Table 5: CD147 expression in the four studied groups**

| Group | | CD147 Scoring | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| Urothelial carcinoma | N | 46 | 23 | 69 |
| | % | 66.7% | 33.3% | 100% |
| Squamous cell carcinoma | N | 10 | 0 | 10 |
| | % | 100% | 0% | 100% |
| Adenocarcinoma | N | 4 | 1 | 5 |
| | % | 80% | 20% | 100% |
| Neuroendocrine carcinoma | N | 1 | 0 | 1 |
| | % | 100% | 0% | 100% |
| Total | N | 61 | 24 | 85 |
| | % | 71.8% | 28.2% | 100% |
| Chi-square | $X^2$ | 48.706 | | |
| | P-value | MC 0.001* | | |

MC: Monte Carlo for Chi square test

**Table 6: The relation between CD147 expression and grading in UC cases**

| Grade | | CD147 Scoring | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| Low Grade | N | 1 | 17 | 18 |
| | % | 5.6% | 94.4% | 100% |
| High Grade | N | 45 | 6 | 51 |
| | % | 88.2% | 11.8% | 100% |
| Total | N | 46 | 23 | 69 |
| | % | 66.7% | 33.3% | 100% |
| Chi-square | $X^2$ | 40.926 | | |
| | P-value | 0.001* | | |

**Table 7: Relation between CD147 expression and muscle invasion in UC cases**

| Stage | | CD147 Scoring | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| Non-muscle invasive | N | 6 | 17 | 23 |
| | % | 26.1% | 73.9% | 100% |
| Muscle-invasive | N | 40 | 6 | 46 |
| | % | 87% | 13% | 100% |
| Total | N | 46 | 23 | 69 |
| | % | 66.7% | 33.3% | 100% |
| Chi-square | $X^2$ | 25.565 | | |
| | P-value | 0.001* | | |

**Table 8: The relation between GATA3 and CD147 expression in urinary bladder carcinoma**

| CD 147 Scoring | | | GATA3 Scoring | | Total |
|---|---|---|---|---|---|
| | | | Positive | Negative | |
| Negative | | N | 24 | 0 | 24 |
| | | % | 40% | 0% | 100% |
| Positive | | N | 36 | 25 | 61 |
| | | % | 60% | 100% | 100% |
| Total | | N | 60 | 25 | 85 |
| | | % | 100% | 100% | 100% |
| Chi-square | $X^2$ | 4.607 | | | |
| | P-value | 0.032* | | | |

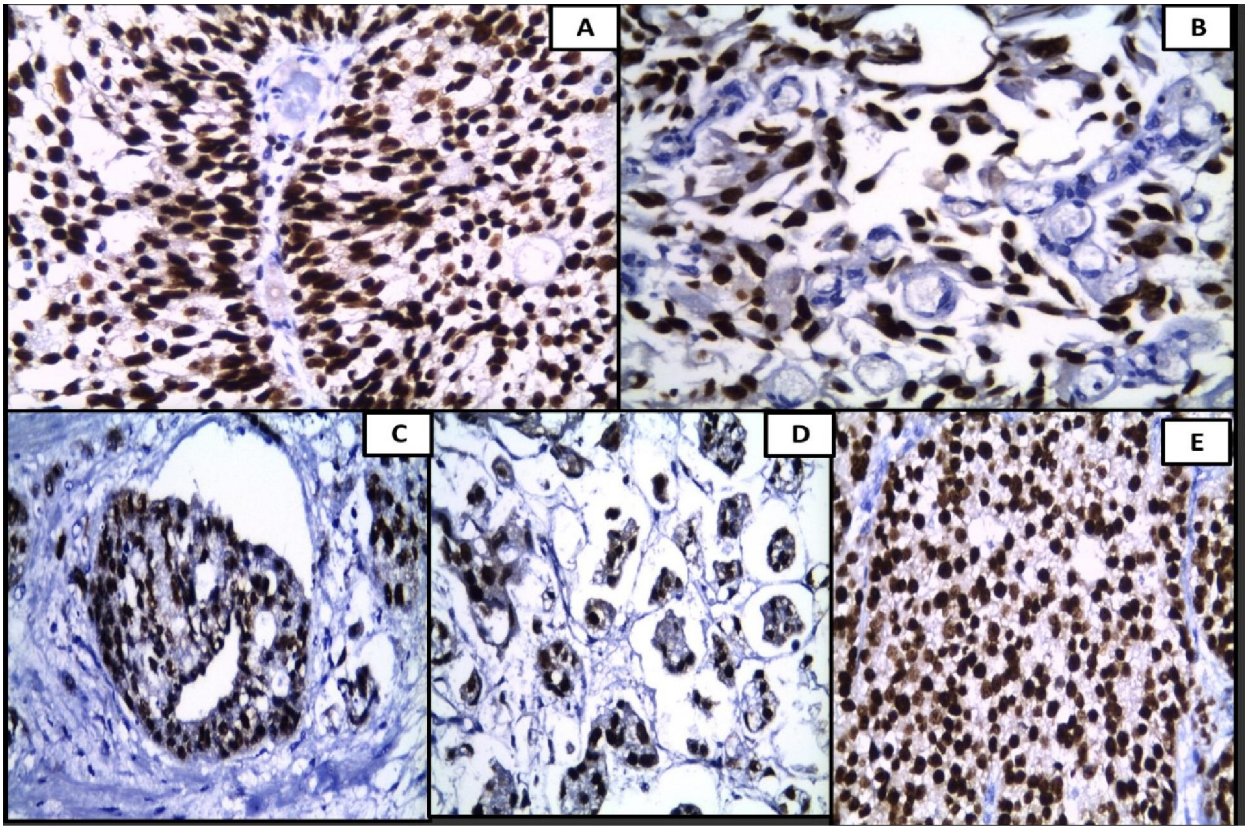


**Figure 1: Low grade (A), Plasmacytoid (B), Microcystic (C), Micropapillary (D), Clear cell UC (E), all showed strong positive nuclear GATA3 expression (+3) (Streptavidin biotin x400).**

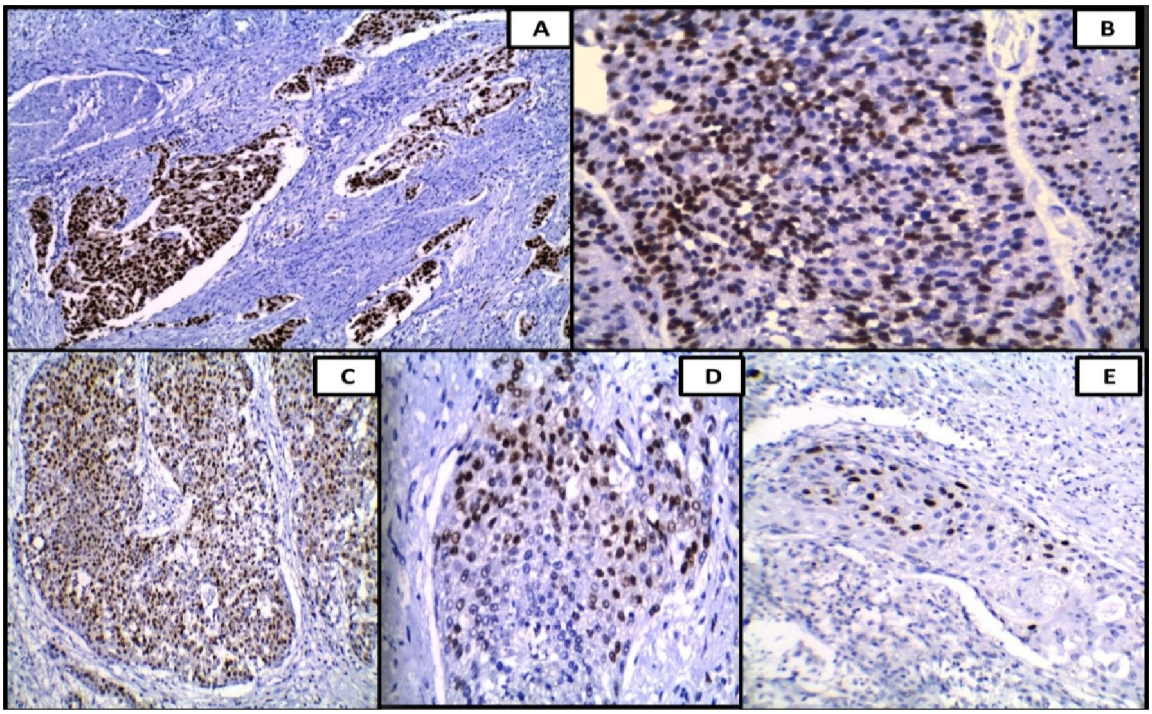


**Figure 2: High grade UC showed strong (+3) (A) (Streptavidin biotin x 200), moderate (+2) (B) (Streptavidin biotin x 400) GATA3 expression, UC with squamous differentiation showed Strong (+3) (C) (Streptavidin biotin x 200), Moderate (+2) (D), Weak (+1) (E) (Streptavidin biotin x 400), GATA3 expression.**

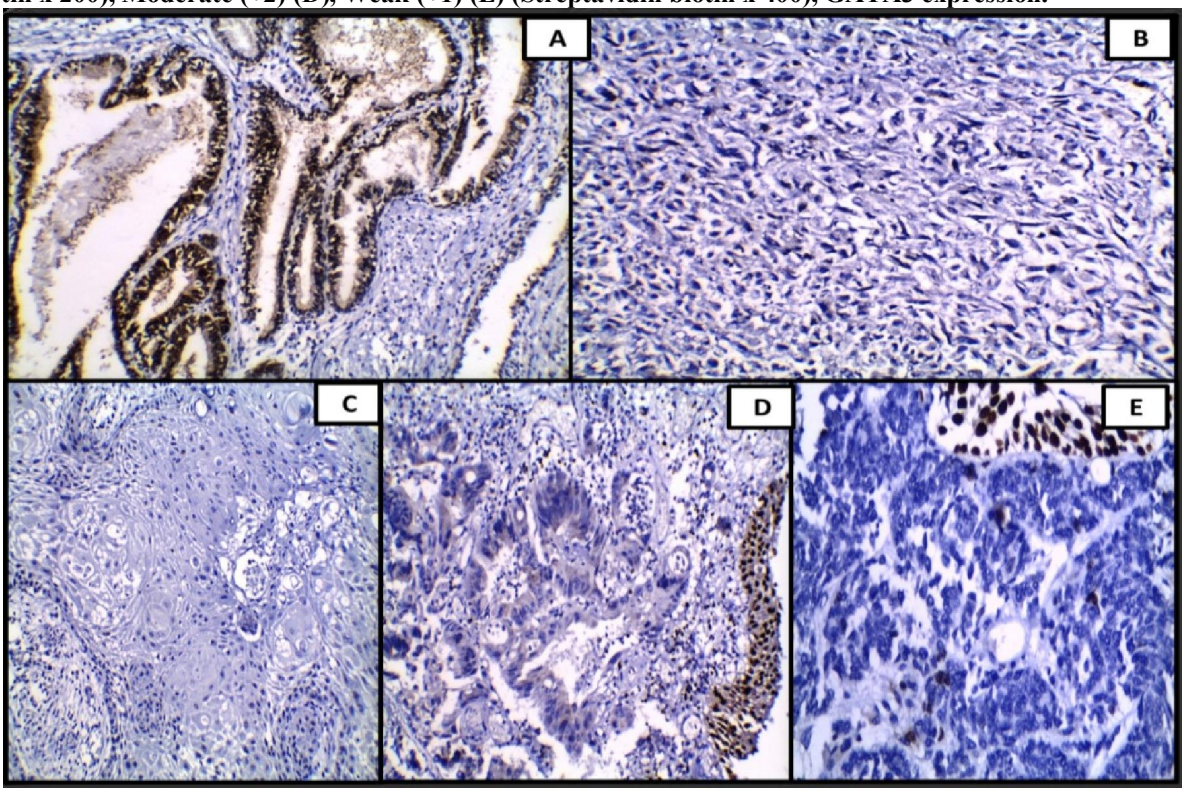


**Figure 3: UC with glandular differentiation showed strong (+3) GATA3 expression (A), Sarcomatoid UC (B), SCC (C), Adenocarcinoma (D), SmCC (E), all showed negative GATA3 expression (Streptavidin biotin x 200).**

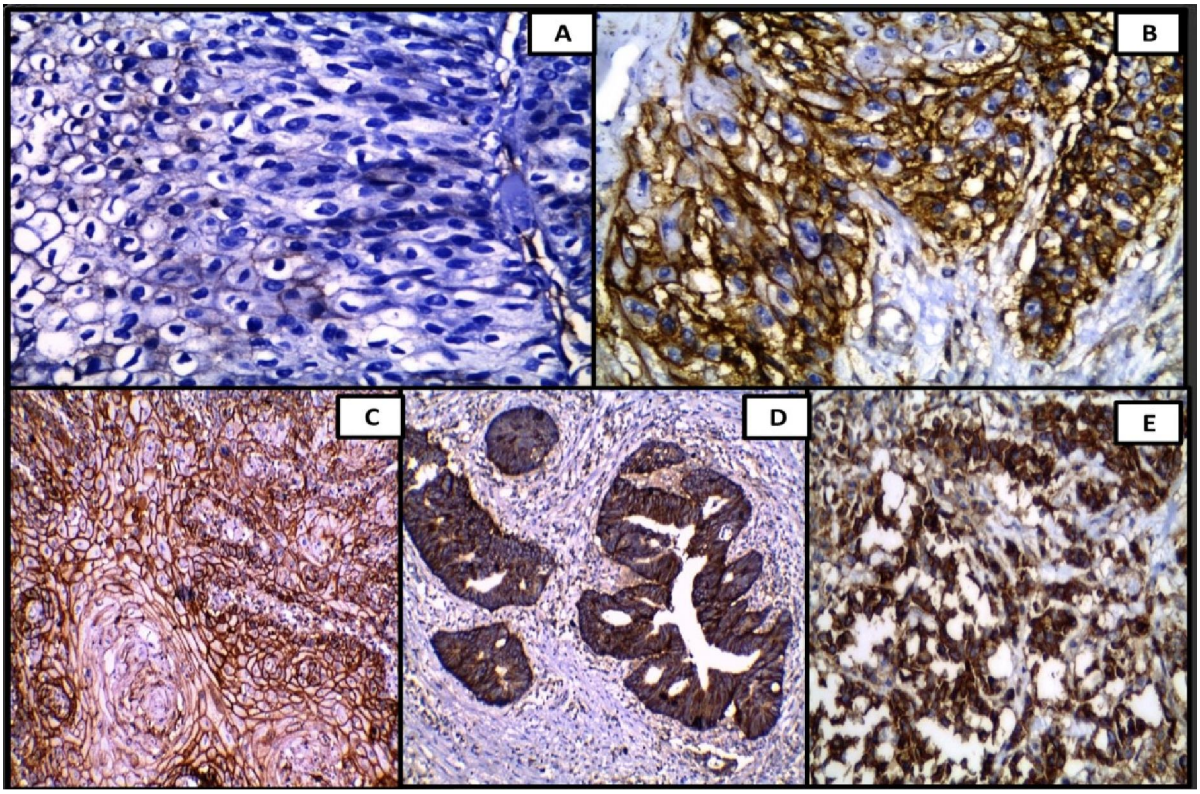


**Figure 4: Low grade papillary UC showed negative CD147 expression (A) (Streptavidin biotin x400), High grade UC (B) (Streptavidin biotin x400), SCC (C), Adenocarcinoma (D), SmCC (E), all showed positive membranous CD147 expression (Streptavidin biotin x200).**

**4. Discussion:**

In the current study, GATA3 exhibited high sensitivity (87%) and specificity (100%) as a diagnostic marker for urothelial carcinoma. This was in agreement with Mohammed et al., (2016) and Ali et al., (2017) who reported high GATA3 sensitivity (70.8%, 89% respectively) and specificity (100%) in the diagnosis of urothelial carcinoma.

Considering UC variants included in our study, strong nuclear GATA3 expression (+3) had been found in all cases of low grade urothelial carcinoma, plasmacytoidurothelial carcinoma, microcysticurothelial carcinoma, micropapillaryurothelial carcinoma, clear cell urothelial carcinoma. This was in agreement with Warrick et al., (2013), Liang et al., (2014), Paner et al., (2014) & Brustmann, (2017) who found that all plasmacytoid, microcystic, micropapillary, clear cell and nested bladder carcinomas that they studied showed strong labeling for GATA3.

GATA3 showed variable expression patterns among UCs with squamous and sarcomatoid differentiation. This was in agreement with Miyamoto et al., (2012) who found that among their 9 cases with squamous differentiation, 56%, 22% and 22% showed 0, +1, and +2 GATA3 positivity, respectively, and the expression levels were significantly lower in these cases than in pure urothelial carcinomas. Lopez-Beltran et al., (2017) reported that UC with squamous differentiation expressed GATA3 in 35% of cases and that no specific markers exist to help with the distinction of squamous differentiation in UC from pure SCC, so, the diagnosis must rely on the clinical history and the absence of a clear-cut conventional UC component upon histologic analysis. Verduin et al., (2015) found that none of their UCs with squamous differentiation showed any reactivity to GATA3 in the squamous component.

Regarding sarcomatoid UC, Chang et al., (2013) & Liang et al., (2014) found also a significant decrease in GATA3 expression in their sarcomatoid UC (31% & 16% respectively). In contrast to these results, Verduin et al., (2015) reported that sarcomatoid UCs showed GATA3 labeling in 66% of cases. Also Zibadi et al., (2014) stated that GATA3 may play an emerging role in distinguishing sarcomatoid carcinoma from inflammatory myofibroblastic tumor (IMT) as the sarcomatous component in their study was positive for GATA3. Monn and Cheng, (2016), reported that while GATA3 expression was similar between micropapillary and pure UC, GATA3 levels have been reported to be significantly lower in other

variants of UC such as squamous differentiation variant and sarcomatoid variant. The lower sensitivity of GATA3 in cases of UC with squamous and sarcomatoid differentiation may be attributed to that they belong to basal-like urothelial carcinoma which are GATA3 negative and CK5/6 positive. That fact will affect the interpretation of GATA3 stains in the context of possible metastasis from primary bladder carcinomas with variant morphologic patterns, as well as their distinction from secondary bladder involvement by tumors of non-urothelial origin.

All SCC cases included in the present study showed GATA3 negative expression. Other studies showed somewhat different results regarding SCC: Liang et al., (2014) & Helmy et al., (2015) found that an overall 7% and 5% of the well-differentiated SCCs were positive for GATA3, respectively, whereas none of the poorly differentiated cases showed any positivity for GATA3, with a significant difference from urothelial carcinoma with squamous differentiation and conventional urothelial carcinoma and concluded that GATA3 can be used in differentiating UC with squamous differentiation from pure SCC.

All adenocarcinoma cases in the current study showed negative GATA3 expression. This result was in agreement with Rao et al., (2013) & Kashyap and Shukla (2017), who found that all their adenocarcinoma cases lacked GATA3 expression and concluded that GATA3 is a useful marker in differentiating urothelial carcinoma with glandular differentiation from primary bladder adenocarcinoma.

All low grade UC cases showed strong GATA3 positivity (+3) (100%), while 82.4% of the high grade cases showed positive GATA3 expression. Regarding the degree of MP invasion in UC cases, 95.7% of NMI cases showed strong GATA3 nuclear positivity (+3), while 82.6% of MI cases showed GATA3 positive nuclear expression. This was in agreement with Miyamoto et al., (2012) who stated that 98% of low-grade tumors were GATA3 positive, whereas 80% of high-grade carcinomas were GATA3 positive. Kosuge et al., (2017) reported that there was a decrease in GATA3 immunoreactivity in higher-stage and higher-grade urothelial carcinomas. Ali et al., (2017) found that GATA3 showed strong positive in 100% pT1 urothelial carcinoma, 64% pT2 urothelial carcinoma and weak expression in 66.6% of pT4 tumors. Inoue et al., (2017) also found that 67% of low grade versus 48% of high grade UCs and 54% of NMI versus 50% of MI UCs were immunoreactive for GATA3. Bahria Sediki et al., (2016) & Rochester et al., (2017) also stated that strong GATA3 expression could be considered as a predictive factor for good patient survival. These results indicated that reduction in GATA3 expression is associated with high-grade and muscle invasive bladder carcinoma.

In the current study, 71.8% of UBC cases showed membranous CD147 positivity: 66.7% of urothelial carcinoma cases showed positive CD147 expression, all SCC cases (100%), 80% of adenocarcinoma cases and the neuroendocrine small cell carcinoma case showed also positive CD147 expression.

This was in agreement with Mujtaba et al., (2017) who reported that positive CD147 expression was shown in 70.69% of their UBC cases. Also, Weidle et al., (2010) showed that CD147 expression was found in 86.8% of their studied cases with the highest incidence was found among squamous cell carcinoma cases (100%). Abd El-Rehim et al., (2013) also found that CD147 expression was detectable in 71.2% of bladder carcinomas and a significantly higher CD147 expression was detected in SCC compared to urothelial carcinoma.

The majority of low grade UC cases were negative for CD147 (94.4%). Meanwhile, 89.6% of the high grade cases showed positive CD147 expression. Regarding muscle invasion in UC cases, the majority of the NMI cases were CD147 negative (73.9%), whereas most MI cases showed positive CD147 expression (88.7%). Positive CD147 staining was significantly associated with high tumor grade and muscle invasion.

These results were in agreement with Xin et al., (2016), Hongru et al., (2017), Horikawa et al., (2017) & Qiao et al., (2018) who stated that positive CD147 staining was significantly associated with advanced TNM stages, poor prognosis, lymph node metastases and high histological grade. Their results indicated that NMI urothelial carcinomas had strong relation with negative or low CD147 expression scores, compared to MI tumors. Similarly, low-grade tumors were associated with negative or low CD147 expression scores, whereas high-grade tumors showed higher expression scores. Huhe et al., (2017) & Todenhöfer et al., (2018) also reported that CD147 expression was significantly associated with lower 5-year disease free survival (DFS) and overall survival (OS) rates.

In two studies made by Dai et al., (2013) & Chen et al., (2015), they found that the subpopulations of tumor cell lines that constitutively expressing high levels of cell-surface CD147 exhibit cancer stem-like cell properties; much greater invasiveness and drug resistance than those expressing low levels of cell-surface CD147. In other studies made by Huhe et al., (2017) & Kendrick et al., (2017), they reported that high CD147 expression may serve roles in tumor progression and may be diagnostic and therapeutic targets in urinary bladder carcinoma. Shang et al.,

(2018) reported that CD147 was demonstrated to be the most frequently up-regulated mRNA and protein in metastatic cells isolated from the bone marrow of patients with cancer and a novel regulator of immune-related genes, suggesting that it serves a key role in tumorigenesis and metastasis.

The relation between GATA3 and CD147 expression in our study was statistically significant. Positive CD147 expression is associated with high grade and stage, while GATA3 positive expression is associated with low grade and stage.

**Conclusions:**

- GATA3 seemed to be a valuable tool for confirming the urothelial origin of microcystic, micropapillary, plasmacytoid and clear cell variants of UC, and exhibited high sensitivity and specificity for bladder carcinoma with purely urothelial differentiation.
- GATA3 could be used as a useful marker in differentiating urothelial carcinoma with glandular differentiation from primary bladder adenocarcinoma, prostatic adenocarcinoma as well as from metastatic carcinomas.
- Lesions showing squamous or sarcomatoid differentiation were particularly challenging, as GATA3 expression was significantly lower in these variants of UC and would require the application of several additional immunostains for more diagnostic accuracy.
- GATA3 was negative in all cases of SCC and adenocarcinoma. Therefore, GATA3 could be used in excluding other non-urothelial tumors.
- Decreased GATA3 expression was associated with high tumor grade and muscularispropria invasion.
- CD147 positive expression was significantly related to high grade and advanced stage of bladder carcinoma.
- There was an inverse relationship between GATA3 and CD147 in bladder carcinoma. CD147 was overexpressed than GATA3 in high grade advanced stage urinary bladder carcinoma.
- Study the possibility of CD147 to be used as a therapeutic target for advanced bladder cancer, based on inhibition of CD147 expression by CD147-specific siRNA, may be of value.

**Declaration of Conflicting Interests**

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