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ORIGINAL RESEARCH

Expression patterns of EGFR, PD-L1, and Ki-67 among Ugandan patients with muscle-invasive bladder cancer

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Introduction: Bladder cancer ranks as the ninth most common cancer globally, with muscle-invasive urothelial carcinoma (MIUC) exhibiting poor survival rates. Biomarkers such as epidermal growth factor receptor (EGFR), programmed death-ligand 1 (PD-L1), and Ki-67 have been studied for their potential in guiding management. However, limited data exists on their expression in Ugandan populations. This study examines the expression of these biomarkers in MIUC patients to provide insights for improved management.

Methods: A descriptive, cross-sectional study was conducted on 52 archived formalin-fixed, paraffin-embedded (FFPE) tissue samples of MIUC patients at Mulago National Referral Hospital (MNRH). Immunohistochemistry (IHC) was performed using the Ventana BenchMark ULTRA system with antibodies against PD-L1, EGFR, and Ki-67. Expression thresholds were set at > 5% for PD-L1, > 10% for EGFR, and > 15% for Ki-67. Data were analysed using RStudio, with associations assessed using Cramér's V matrix.

Results: Among the 52 samples, Ki-67 showed the highest expression (55.8%), followed by EGFR (13.5%) and PD-L1 (5.8%). Most associations between categorical variables were weak, with a moderate association demonstrated between variant histology and lymphovascular invasion (LVI).

Conclusion: This study highlights the low expression of PD-L1 and EGFR in contrast to the high expression of Ki-67. These findings emphasise the importance of considering Ki-67 as a potential biomarker for aggressiveness in urothelial carcinoma (UC). The low PD-L1 expression raises questions about the applicability of immunotherapy in Ugandan populations. Future research with larger cohorts and genomic analysis is recommended.

Keywords: muscle-invasive bladder cancer, biomarkers, EGFR, PD-L1, Ki-67

Introduction

Bladder cancer is the ninth most diagnosed cancer globally, with 614 298 registered new cases in 2022, a 7.1% increase from 2020.[1] Among men, it ranks sixth, with 523 674 cases, accounting for 5.4% of all male cancer cases.[2] Urothelial carcinoma (UC), the most common bladder malignancy, presents either as a non-muscle-invasive or muscle-invasive disease. The former comprises the bulk (70–85%) of patients in most countries.[3] However, in Uganda, most patients (58.9%) present with the muscle-invasive type.[4] Muscle-invasive urothelial carcinoma (MIUC) is known to have an extremely low five-year overall survival rate of 10–20%.[5,6]

Through immunohistochemistry (IHC), biomarkers such as epidermal growth factor receptor (EGFR), programmed death-ligand 1 (PD-L1), and Ki-67 have been investigated and proposed for use in diagnosing and developing pharmaceuticals for bladder cancer.[7,8] EGFR belongs to the ErbB tyrosine-kinase receptor family, which modulates cell growth, differentiation, and proliferation.[9] Nearly 74% of bladder cancer tissue specimens express EGFR, although low expression is observed in normal bladder tissue.[10] This observation influenced the development of prospective anti-EGFR targeted therapies such as panitumumab, lapatinib, cetuximab, and gefitinib.[11]

Conversely, PD-L1 is a transmembrane protein expressed by cytotoxic T cells and other immune cells. To avoid the action of these T cells, normal cells express a programmed death receptor (PD-1) that binds to PD-L1, thereby enhancing immune system modulation. Unfortunately, urothelial cancer cells also express PD-L1, allowing them to evade the body's immune response.[12,13] This revelation led to the development of drugs such as pembrolizumab, atezolizumab, nivolumab, durvalumab, and avelumab, which block the PD-L1/PD-1 interaction, known as immune checkpoint inhibitors. However, several studies from different regions have reported varying positivity rates of PD-L1, ranging from 20% to 65%.[5,14]

Ki-67 is a nuclear protein of molecular mass 359 kDa, coded by the gene on chromosome 10. It is expressed in all phases of the cell cycle except the resting phase, and it is a significant marker of tumour proliferation. An increase in its expression is associated with cell growth and is thus commonly used as a marker for quantifying proliferating cells.[15] The Ki-67 index is a crucial prognostic and predictive marker for aggressive tumours, i.e. a high Ki-67 index (approximately ≥ 20%) is associated with a poor prognosis.[16]

Although a significant number of patients with MIUC may not benefit from immunotherapeutic drugs, they have several advantages over conventional chemotherapy.[13] Due to their high costs, these drugs are unavailable at the Uganda Cancer Institute, the only dedicated

national referral cancer centre. Furthermore, there are no local data or published protocols available in any of the other five East African countries regarding management. We thus investigated the frequency of expression of three key biomarkers (EGFR, PD-L1, and Ki-67) to generate results that will guide further studies, inform policymakers on the allocation of funds for such drugs, and ultimately translate into improved patient outcomes.

Methods

A descriptive, cross-sectional study was conducted at the Mulago National Referral Hospital (MNRH) complex. We consecutively included 52 archived FFPE tissue blocks that we re-confirmed to have MIUC from 2020 to 2022. All specimens were obtained through transurethral resection. The histopathologic staging was performed per the 2004 World Health Organization (WHO) classification.[17] Damaged tissue blocks with < 50% viable tumour, those with extensive necrosis, prior exposure to radiotherapy or chemotherapy, and missing vital demographic data were excluded.

While using a Ventana BenchMark ULTRA system, different 4 µm thick tissue sections were stained following the manufacturer's protocols for the primary rabbit monoclonal anti-EGFR antibody (clone no. 5B7; cat no. 790–4347, Ventana Medical Systems, Inc., Tucson, United States), Ventana PD-L1 (SP142; A7020), and anti-Ki-67 (clone no. 30-9; cat no. 790–4286). Reaction products were visualised using a diaminobenzidine IHC detection kit, and subsequently, sections were counterstained with Harris Haematoxylin.

Tumour cell (TC) staining intensity > 10% was considered positive for EGFR and > 5% for PD-L1.[18-20] For Ki-67, nuclear staining at a > 15% rate of TCs was considered positive.[21] Tonsil tissue was used as a positive control for Ventana anti-PD-L1 (SP142) and anti-Ki-67 (30-9), while placental tissue was used as a control for Ventana anti-EGFR. Two consultant pathologists independently examined the tissues without knowledge of the clinical data. In the event of a disagreement, a consensus was reached by re-examining the tissues under a multiheaded microscope.

Data analysis and presentation

Data was collected using a questionnaire, entered into REDCap (Research Electronic Data Capture), and later exported as a comma-separated values (CSV) file into RStudio for analysis. Continuous data were summarised as the mean with standard deviation. Categorical data were summarised as frequencies and proportions and presented in table and figure formats. Associations among categorical variables (sex, variant histology, LVI, EGFR, PD-L1, and Ki-67) were determined by Cramér's V association matrix. The interpretation scale is as follows: 0 to ≤ 0.3 indicates a weak association, 0.3 to ≤ 0.6 indicates a moderate association, and 0.6 to 1 indicates a strong association.

Results

Clinicopathologic characteristics of patients

Overall, 52 participant samples were included in the study. All patients presented with haematuria and a bladder mass, as seen on imaging. The participants' mean age and standard deviation were 59.3 ± 12.9 years. The majority were males (31/52, 59.6%). Most samples exhibited variant morphologies (28/52, 53.8%). The majority had LVI (43/52, 83%). The highest immunoreactivity was seen with Ki-67 (28/52, 55.8%) (Table I, Appendix 1).

Table I: Immunoreactivity of Ki-67, EGFR, and PD-L1

Biomarker	Positive (%)	Negative (%)
Ki-67	29 (55.8)	23 (44.2)
EGFR	7 (13.5)	45 (86.5)
PD-L1	3 (5.8)	49 (94.2)

Associations between different categorical variables

The values ranged from 0 (no association) to 1 (perfect association) (Figure 1). Variant histology and LVI exhibit a moderate association

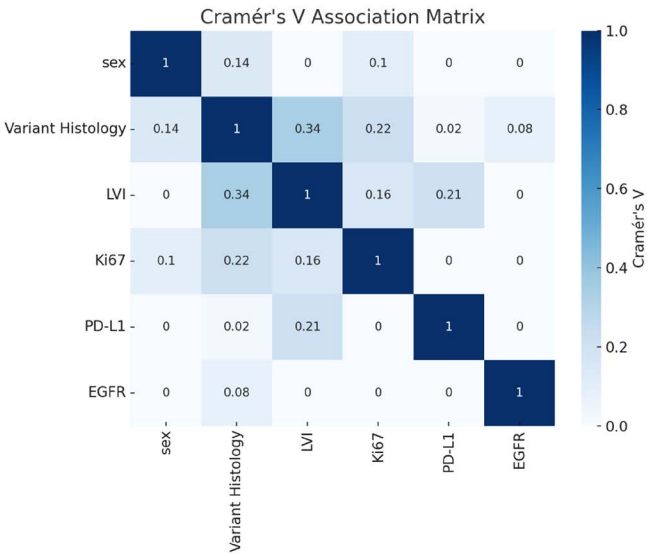

Figure 1: Cramér's V association matrix of the study's categorical variables

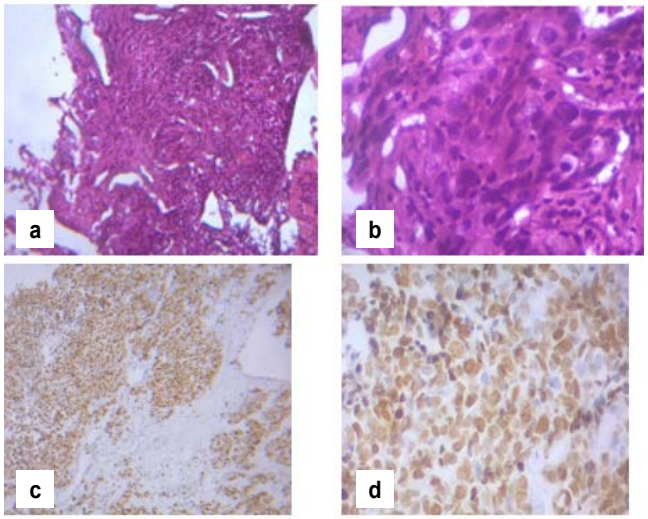

Figure 2: Immunoreactivity of Ki-67, muscle-invasive urothelial carcinoma with squamous differentiation
Image a (× 60) – tumour extending into the detrusor muscle (≥ pT2), solid papillary nests of dysplastic urothelium infiltrating and inciting desmoplastic stromal reaction.
Images a (× 60) and b (× 100) – prominent lymphovascular and perineural invasion, increasing the likelihood of regional nodal metastasis and tumour extension beyond the bladder wall.
Images c (× 10) and d (× 40) – strong Ki-67 immunopositivity.

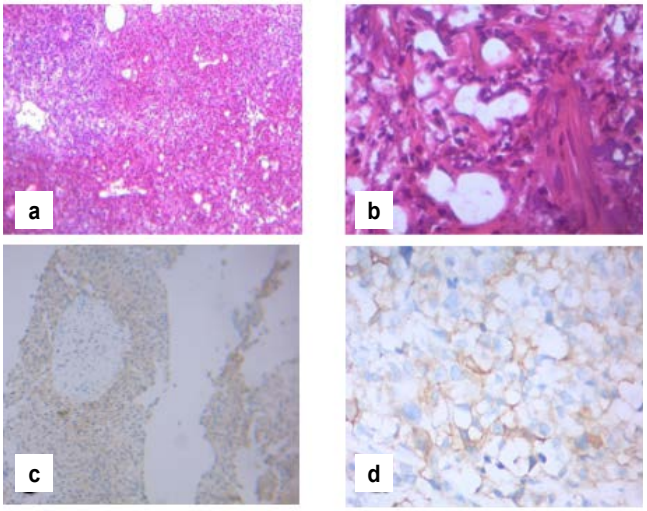

Figure 3: Immunoreactivity of EGFR, muscle-invasive urothelial carcinoma, plasmacytoid variant
Image a (× 60) – polypoid projections comprised of urothelium with plasmacytoid features.
Image b (× 100) – tongue-like projections through the detrusor muscle (≥ pT2).
Images c (× 10) and d (× 40) – no LVI, entailing less likelihood of regional nodal metastasis. Moderate immunopositivity of EGFR.

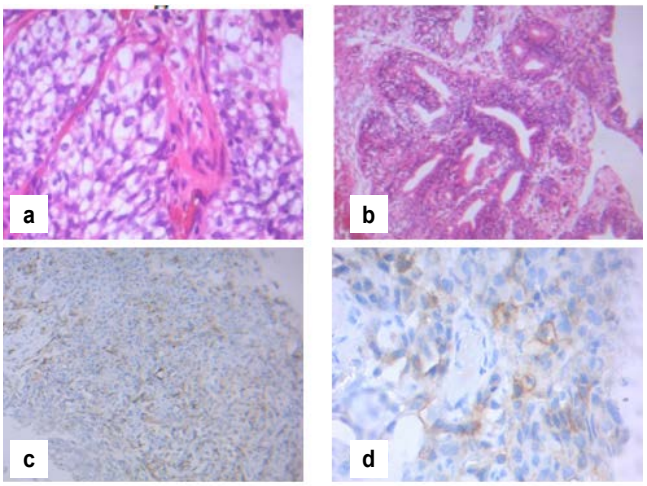

Figure 4: Immunoreactivity of PD-L1, muscle-invasive urothelial carcinoma, lipid-rich variant
Images a (× 60) and b (× 100) – lipid-rich features comprise 80%, and micropapillary features comprise 20%. There is the presence of carcinoma in situ. Lamina propria and muscularis propria are invaded. There is angiolymphatic invasion (≥ pT2), increasing the likelihood of regional nodal metastasis.
Images c (× 10) and d (× 40) – moderate immunopositivity of PD-L1.

(0.34). Weak associations (0–0.3) were observed between variant histology and Ki-67 (0.22) and between LVI and PD-L1 (0.21). Generally, PD-L1 displays minimal interaction with most factors. Sex and EGFR demonstrated weaker or no associations (values close to 0) with most other variables (Figure 2-4).

Discussion

The results of this study indicate a low PD-L1 (5.8%) and EGFR (13.5%) expression, with a high Ki-67 (55.8%) expression. The latter is a known proliferation biomarker that is highly expressed in aggressive cancers.[22] As a general principle, MIUC is intrinsically aggressive; however, certain MIBC tumours may not exhibit Ki-67 positivity.[23] That is to say, Ki-67 overexpression is associated with an advanced pathologic stage, higher grade, LVI, and metastases to lymph nodes.[24,25] However, not all the listed factors accompany a high Ki-67. For instance, Ki-67 and PD-L1 showed a weak association with LVI, which is consistent with a systematic review and meta-analysis of 5 147 bladder cancer patients.[26] Additionally, with more than 50% Ki-67 expression, our study aligns with documented findings elsewhere.[26] Therefore, this highlights the importance of considering Ki-67 expression analysis as an adjunctive biomarker of aggressiveness in routine diagnostic and therapeutic decision-making processes for patients with UC.

Positive Ki-67 expression is strongly associated with positive PD-L1 expression.[6] Therefore, given the high expression of Ki-67 in this study, it was expected that PD-L1 would also be highly expressed, a situation that we did not observe, perhaps due to the wide intratumor genetic heterogeneity exhibited by UC. Notably, the results of PD-L1 expression rates from several studies have generally been inconsistent due to the vast heterogeneity of UC and the use of different PD-L1 clones and scoring systems.[2,27] Our study found a considerably low expression of PD-L1 (5.8%), a finding also observed in several other studies. For instance, in a study in Lebanon, only 5/54 (9%) cystectomy specimens with confirmed muscle-invasive bladder cancer were PD-L1-positive.[28]

Conversely, some studies that used the exact clone (SP142) and the same cut-off (5% TC) as our study reported a high PD-L1 positivity rate (i.e. 23%).[27] Similarly, from the clinical trials that led to the Food and Drug Administration (FDA) approval of atezolizumab, a 27% positivity rate was reported using the SP142 clone.[29] Lebanon is reported to have one of the highest incidences of UC in the world; a 43% positivity rate was reported using a ≥10 cut-off with the 22C3 clone among 101 tumour specimens.[24] Similarly, a retrospective study of 604 cystectomy specimens from Peking University First Hospital in Beijing, China, reported a 61.2% positivity rate for PD-L1.[30]

Although some studies report no correlation, the importance of PD-L1 expression in determining the clinical outcome of patients is unclear.[26,31] However, a substantial number of studies reported better responses to immune checkpoint inhibitors (ICIs)in patients whose immune cells express PD-L1.[32,33] Likewise, tumours that stained positive for PD-L1 (≥ 10) and had ≥ 5% expression in the KEYNOTE-052 and KEYNOTE-361 studies showed a better response to treatment.[2,34] Considering our population has low PD-L1 expression, it raises questions about how our patients can benefit from immunotherapy, thereby providing opportunities for further *studies.*

Another potential cell surface therapeutic target is the EGFR, which is overexpressed in about 74% of UC tissue specimens.[35] Moreover, its localisation in urothelial cells' basal and luminal layers makes intravesical therapy a potential treatment option.[36] Additionally, EGFR is an independent predictor of poor survival and disease progression in bladder cancer.[37] Several previous IHC studies reported high but different positivity rates (90%, 86%, 74%, 46%, and 35%).[35,38-41] Our study shows that the frequency of EGFR expression is considerably lower (14%) than the above figures. However, The Cancer Genome Atlas (TCGA) project indicated similar expression frequency rates of up to 11% for MIUC.[43] A better

outcome for patients with UC treated with EGFR inhibitors (gefitinib or erlotinib) is seen in those with positive EGFR than those with negative expression.[44] Our findings raise further questions about whether our patients can benefit from such molecules.

Study limitations

Our study, while insightful, has limitations. A modest sample size was used from a single tertiary hospital, introducing potential selection bias. However, it can be argued that MNRH manages the most significant number of these patients in the country. Furthermore, comparing our results with those of previous studies was challenging due to the variations in methodologies and clones used. Despite these constraints, our findings provide valuable insights into the EGFR, PD-L1, and Ki-67 biomarkers in our population.

Conclusion

The study found low PD-L1 and EGFR expression but high Ki-67 levels in UC, suggesting that Ki-67 could serve as a marker for tumour aggressiveness. The low PD-L1 expression questions the relevance of immunotherapy in Ugandan patients. More extensive studies, including those with genomic analysis, are needed to validate these findings.

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Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

Clearance was obtained from the Makerere University School of Medicine research and ethics committee under protocol reference number Mak-SOMREC-2021-257.

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Appendix 1

Immunoreactivity results of Ki-67, PD-L1, and EGFR

Sample number	Sex	Age (years)	Variant histology	LVI	Ki-67	PD-L1	EGFR
1	F	30	No	LV+	Positive	Negative	Negative
2	M	70	Yes	LV+	Positive	Negative	Positive
3	M	52	Yes	LV+	Negative	Negative	Positive
4	M	66	Yes	LV+	Negative	Negative	Negative
5	F	70	Yes	LV+	Positive	Negative	Negative
6	M	76	No	LV+	Positive	Negative	Negative
7	M	52	No	LV+	Negative	Negative	Negative
8	M	47	No	LV-	Negative	Negative	Negative
9	M	56	Yes	LV+	Positive	Negative	Positive
10	M	74	No	LV-	Positive	Negative	Negative
11	M	51	No	LV+	Positive	Negative	Negative
12	M	57	No	LV+	Positive	Negative	Negative
13	M	49	No	LV-	Negative	Positive	Negative
14	M	50	No	LV+	Positive	Negative	Negative
15	M	70	Yes	LV+	Negative	Negative	Negative
16	M	59	Yes	LV+	Positive	Negative	Negative
17	F	67	No	LV-	Negative	Negative	Positive
18	M	42	Yes	LV+	Positive	Negative	Negative
19	F	42	No	LV-	Negative	Negative	Negative
20	F	62	Yes	LV+	Positive	Negative	Negative
21	F	57	No	LV+	Negative	Negative	Negative
22	F	54	Yes	LV+	Positive	Negative	Negative
23	M	47	Yes	LV+	Negative	Negative	Negative
24	F	66	No	LV+	Negative	Negative	Positive
25	F	41	Yes	LV+	Negative	Negative	Negative
26	F	56	Yes	LV+	Negative	Negative	Negative
27	F	63	No	LV+	Negative	Negative	Negative
28	F	50	No	LV-	Negative	Negative	Negative
29	M	56	Yes	LV+	Positive	Negative	Negative
30	M	70	Yes	LV+	Positive	Negative	Negative
31	M	98	No	LV+	Negative	Negative	Negative
32	F	56	Yes	LV+	Positive	Negative	Negative
33	M	46	Yes	LV+	Positive	Negative	Negative
34	M	70	Yes	LV+	Positive	Negative	Negative
35	M	63	Yes	LV-	Positive	Negative	Negative
36	M	70	No	LV-	Negative	Negative	Negative
37	M	55	Yes	LV+	Negative	Negative	Negative
38	M	49	Yes	LV+	Positive	Positive	Negative
39	M	75	Yes	LV+	Positive	Negative	Negative
40	F	56	No	LV+	Positive	Negative	Negative
41	F	46	No	LV-	Positive	Positive	Negative
42	F	66	No	LV+	Negative	Negative	Negative
43	F	45	Yes	LV+	Positive	Negative	Positive
44	M	87	No	LV+	Negative	Negative	Negative
45	F	85	Yes	LV+	Positive	Negative	Negative
46	F	56	No	LV+	Negative	Negative	Negative
47	M	61	No	LV+	Positive	Negative	Negative
48	F	51	Yes	LV+	Negative	Negative	Negative
49	M	60	Yes	LV+	Negative	Negative	Negative
50	F	49	No	LV+	Positive	Negative	Negative
51	M	58	Yes	LV+	Positive	Negative	Positive
52	M	79	Yes	LV+	Positive	Negative	Negative

EGFR – epidermal growth factor receptor, F – female, Ki-67 – , LVI – lymphovascular invasion, M – male, PD-L1 – programmed death-ligand 1