Original Article

OPEN ACCESS

Expression of p53 as a Prognostic Marker in low and high-grade Urothelial Carcinoma: A Cross-Sectional Analysis

Iqra Butt[1], Henna Azmat[2], Zainab Taj[3], Uzma Meral[4], Ghazal Zaffar[5], Javaria Ahsan[6],

[1]Senior Lecturer Pathology, CMH Medical College, Kharian
[2]Associate Pathology, Federal Govt Plyclinic PGMI, Islamabad
[3]Registrar Hisopathology dept, FUMC, Islamabad, [4]Assistant Professor, CMH Medical College, Kharian
[5]Assistant Professor Pathology, CMH Medical College, Kharian,
[6]Associate Professor Pathology, CMH Medical College, Kharian

Author`s Contribution

[1,6]Substantial contributions to the conception or design of the work; or the acquisition, [3,5]Drafting the work or revising it critically for important intellectual content [2,4]Active participation in active methodology

Funding Source: None
Conflict of Interest: None

Received: April 12, 2024
Accepted: Nov 11, 2024

Address of Correspondent
Dr. Iqra Butt
Senior Lecturer Pathology, CMH Medical College, Kharian
Dr.iqra@gmail.com

ABSTRACT

Objective: To assess the expression of p53 and its correlation with histologic grade and tumor invasiveness in urothelial carcinoma.

Methodology: This cross-sectional study was conducted at the Department of Pathology, Pakistan Institute of Medical Sciences (PIMS), Islamabad, over duration of 24 months August 2018 to August 2020. A total of 96 cases of histologically confirmed urothelial carcinoma were analyzed using immunohistochemistry for p53 expression. p53 positivity was evaluated at 10% and 40% nuclear cut-offs, and further classified into wild-type and aberrant phenotypes. Associations with tumor grade, lamina propria invasion, and muscularis propria invasion were assessed using chi-square tests. The data were analyzed using SPSS version 21. A p-value less than 0.05 was considered statistically significant.

Results: High-grade tumors showed significantly higher p53 expression at both 10% (84.6%) and 40% (63.5%) cut-offs compared to low-grade tumors (45.5% and 22.7%, respectively) ($p < 0.001$). Aberrant phenotypes were more frequent in high-grade tumors (69.2%) versus low-grade (25%) ($p < 0.001$). p53 positivity was also significantly associated with lamina propria and muscularis propria invasion ($p < 0.001$).

Conclusion: p53 immunoexpression correlates strongly with tumor grade and invasion, highlighting its value as a prognostic marker in urothelial carcinoma.

Keywords: Urothelial carcinoma, p53, Immunohistochemistry, Tumor grade, Invasion, Prognostic marker

Cite this article as: Butt I, Azmat H, Taj Z, Meral U, Zaffar G, Ahsan J. Expression of p53 as a Prognostic Marker in low and high-grade Urothelial Carcinoma: A Cross-Sectional Analysis. Ann Pak Inst Med Sci. 2024; 20(4):981-985. doi. 10.48036/apims.v20i4.743.

Introduction

Urothelial carcinoma, previously known as transitional cell carcinoma, is the most prevalent malignancy of the urinary tract and arises from the transitional epithelium lining the renal pelvis, ureters, urinary bladder, and urethra[1]. Among these sites, the urinary bladder is the most frequently involved organ, making bladder cancer one of the leading urological cancers globally.[2]

According to global cancer statistics, bladder cancer ranks as the ninth most common malignancy worldwide, with a notable male predominance[3]. Locally, in Pakistan, urothelial carcinoma remains a significant public health concern, ranking among the top five malignancies in men.[4] Most cases present in the elderly population, particularly between the ages of 50 and 80 years, and the majority are diagnosed at a high histologic grade and with invasion into the bladder wall.[5]

Histologically, urothelial tumors are categorized by the World Health Organization/International Society of Urologic Pathology (WHO/ISUP) grading system into low-grade and high-grade carcinomas.[6] This classification plays a critical role in clinical decision-making, as high-grade tumors are associated with muscle invasion, rapid progression, and poorer prognosis, whereas low-grade tumors tend to recur but rarely progress.[7] However, despite the usefulness of histological grading, it does not always correlate reliably with clinical outcomes.[8] This discrepancy has prompted the search for reliable molecular biomarkers that can predict tumor behavior and guide treatment.[9]

Among the most studied biomarkers in cancer biology is p53, a tumor suppressor protein encoded by the TP53 gene.[10] p53 plays a central role in cell cycle regulation, apoptosis, and DNA repair[11]. Mutations in the TP53 gene are among the most frequent genetic alterations observed in human cancers, including urothelial carcinoma.[12] These mutations often result in the accumulation of abnormal (aberrant) p53 protein in the nucleus, which can be detected immunohistochemically.[13]

Immunohistochemistry (IHC) for p53 expression is widely utilized as a surrogate marker for TP53 mutation status.[14] Notably, increased p53 immunoreactivity has been associated with high-grade tumors and deeper invasion, reflecting a more aggressive phenotype.[15]

Recent studies have proposed that classifying p53 expression into wild-type versus aberrant phenotypes (including null and overexpressed forms) may better reflect the underlying mutational status and correlate more accurately with tumor behavior than simple percentage-based cut-offs.[16] Furthermore, p53 overexpression has been linked to chemotherapy resistance, making it not only a prognostic but also a potentially predictive biomarker in treatment planning[17]. Despite the global interest in p53 as a prognostic tool, limited local data exist regarding the pattern and prognostic relevance of p53 immunoexpression in urothelial carcinoma among Pakistani patients.[18]

This study addresses the paucity of regional data on p53 immunoexpression patterns in urothelial carcinoma and aims to evaluate its association with tumor grade and depth of invasion, thereby assessing its prognostic utility in Pakistani patients.

Methodology

This cross-sectional study was conducted at the Department of Pathology, Pakistan Institute of Medical Sciences (PIMS), Islamabad. The research was carried out over duration of 24 months, from August 2018 to August 2020. Ethical approvaed was obtained from SZABMU IRB no:F-1/2015/ERB/SZABMU/182

The sample size for the study was determined using OpenEpi software, based on an expected frequency of p53 expression in high-grade urothelial carcinoma at approximately 50%, with a confidence interval of 95%, and margin of error of 10%. The minimum calculated sample size was 96 cases, which were included consecutively as they met the inclusion criteria during the study period.

A non-probability consecutive sampling technique was employed. All suitable cases of urothelial carcinoma diagnosed during the study period and fulfilling the inclusion criteria were selected.

All formalin-fixed, paraffin-embedded (FFPE) tissue samples from transurethral resection of bladder tumor (TURBT) specimens or cystectomy specimens diagnosed histologically as urothelial carcinoma were included. Inadequate biopsies and specimens with poor tissue preservation or lacking sufficient tumor tissue were excluded.

Each specimen was processed and stained using standard hematoxylin and eosin (H&E) techniques. Tumors were graded into low-grade and high-grade categories based on the 2016 WHO/ISUP classification system. Invasion into lamina propria and muscularis propria was assessed histologically.

Immunohistochemistry (IHC) was performed using the p53 monoclonal antibody on 3-4 μm thick tissue sections. Slides underwent deparaffinization, rehydration, antigen retrieval, and incubation with the primary antibody followed by application of the secondary antibody and visualization with chromogen. Appropriate positive and negative controls were included for validation.

The immunohistochemical expression of p53 was evaluated by assessing nuclear positivity at two distinct cut-off levels: 10% and 40%. Additionally, p53 expression was interpreted using phenotypic classification, which categorized staining patterns into three groups. Wild-type phenotype was characterized by focal nuclear positivity in 10-60 percent of tumor cell, normal p53 expression. The null phenotype was also described as having no residues stained of the nucleus, which is suggestive of loss-of-function mutations. Finally, the combination of strong diffuse nuclear staining above 60 percent in most tumor cells was the sign of the overexpression phenotype which indicates possible mutation and attainment of p53 gene. This whole method enabled a finer assessment of p53 expression in relation to tumor behavior.

A structured proforma was used to document the data. The variables were age, gender, Tumor grade, depth of invasion and immunohistochemical p53 expression details. The data were analyzed using SPSS version 21. Chi-square test was applied to evaluate the association between p53 expression and tumor grade and invasion. A p-value less than 0.05 was considered statistically significant.

Results

A total of 96 histologically confirmed cases of urothelial carcinoma were included in the study, with a mean age of 64.1 ± 11.1 years (range: 36–86 years). The sample showed a marked male predominance with 75 males (78.1%) and 21 females (21.9%), resulting in a male-to-female ratio of 3.6:1. Based on the WHO/ISUP 2016 classification, 44 cases (45.8%) were categorized as low-grade, while 52 cases (54.2%) were high-grade. Low-grade tumors predominantly displayed non-invasive features, whereas high-grade tumors were frequently associated with deeper invasion into the lamina propria and muscularis propria. The expression of p53 at a 10% nuclear positivity cut-off revealed statistically significant differences between tumor grades, as illustrated in Figure 1. Among the low-grade tumors, 20 cases (45.5%) were p53-positive and 24 cases (54.5%) were negative. In contrast, 44 high-grade tumors (84.6%) showed positive nuclear p53 staining, with only 8 cases (15.4%) being negative. The association between p53 expression and tumor grade was found to be highly significant ($p < 0.001$). These findings suggest that increased p53 expression correlates with higher tumor grade, likely reflecting underlying TP53 gene mutations associated with tumor progression and poor prognosis.

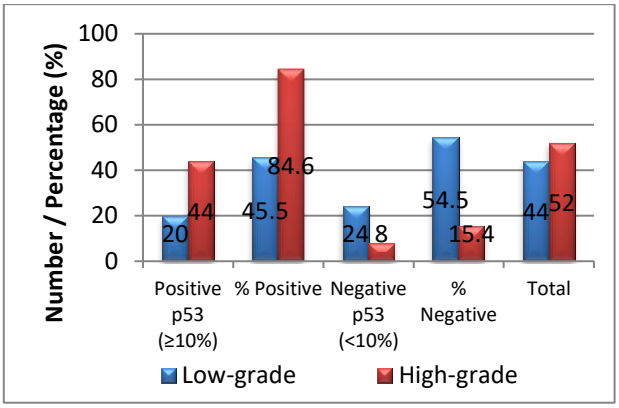

Figure 1. p53 Expression at 10% Cut-off and Its Association with Tumor Grade.

When p53 expression was re-evaluated using a 40% cut-off, the pattern remained statistically significant as shown in Table 1, among low-grade tumors, only 10 cases (22.7%) were p53-positive, while 34 cases (77.3%) were negative. Conversely, 33 high-grade tumors (63.5%) demonstrated positive p53 expression, and 19 cases (36.5%) showed negativity. This difference was also statistically significant with a p-value of less than 0.001. The higher percentage of p53 positivity in high-grade tumors reaffirms its role in aggressive tumor biology. These findings are consistent with global literature and emphasize that increasing p53 expression correlates with worsening tumor differentiation.

The relationship between p53 expression and lamina propria invasion was examined using the 10% positivity threshold. As shown in Table II, among 78 cases with lamina propria invasion, 61 cases (78.2%) were p53-positive and 17 cases (21.8%) were negative. In contrast, of the 18 cases without lamina propria invasion, only 3 cases (16.7%) were p53-positive, and the remaining 15 cases (83.3%) were negative. The association was highly statistically significant ($p < 0.001$), indicating that p53 expression is considerably elevated in tumors that have invaded the lamina propria. This finding supports the hypothesis that p53 overexpression is linked to early invasive potential in urothelial carcinomas. It further suggests that p53 IHC can aid in risk stratification even among non-muscle-invasive tumors.

Table I: p53 Expression at 40% Cut-off and Its Association with Tumor Grade.

Tumor Grade	Positive p53 (≥40%)	Negative p53 (<40%)	Total	p-value
Low-grade	10 (22.7%)	34 (77.3%)	44	
High-grade	33 (63.5%)	19 (36.5%)	52	**<0.001**

Table II: Association between p53 expression (≥10%) and Lamina Propria invasion.

Lamina Propria Invasion	Positive p53 (≥10%)	Negative p53 (<10%)	Total	p-value
Present	61 (78.2%)	17 (21.8%)	78	
Absent	3 (16.7%)	15 (83.3%)	18	**<0.001**

Table III: Association between p53 expression (≥40%) and Muscularis Propria invasion.

Muscularis Propria Invasion	Positive p53 (≥40%)	Negative p53 (<40%)	Total	p-value
Present	28 (84.8%)	5 (15.2%)	33	
Absent	15 (21.4%)	55 (78.6%)	63	**<0.001**

Similarly, a strong correlation was observed between p53 expression at the 40% cut-off and muscularis propria invasion. Among the 33 tumors that invaded the muscularis propria, 28 cases (84.8%) were positive for p53, while 5 cases (15.2%) were negative, as shown in Table III. On the other hand, out of 63 non-invasive tumors, only 15 cases (21.4%) were positive, and 55 (78.6%) were negative. The association was again statistically significant ($p < 0.001$), demonstrating that p53 expression increases with depth of tumor invasion. These results align with existing research linking p53 mutation with poor prognosis, aggressive growth, and

deep tissue involvement. The high expression of p53 in invasive tumors underlines its potential utility in predicting tumor aggressiveness pre-operatively.

To further refine the prognostic utility of p53, its phenotypic classification was analyzed. As illustrated in Figure 2, among the 44 low-grade tumors, 33 cases (75%) exhibited a wild-type staining pattern, whereas 11 cases (25%) showed aberrant patterns (null or overexpression). In contrast, of the 52 high-grade tumors, only 16 cases (30.8%) were wild-type, and 36 cases (69.2%) displayed aberrant p53 expression. This distribution was statistically significant ($p < 0.001$), indicating a strong association between aberrant p53 phenotype and higher tumor grade. These findings suggest that the phenotypic classification of p53 may offer a more accurate reflection of TP53 mutation status than percentage-based cut-offs alone. Incorporating this approach could enhance diagnostic precision in pathology labs with access to immunohistochemistry but limited genetic profiling.

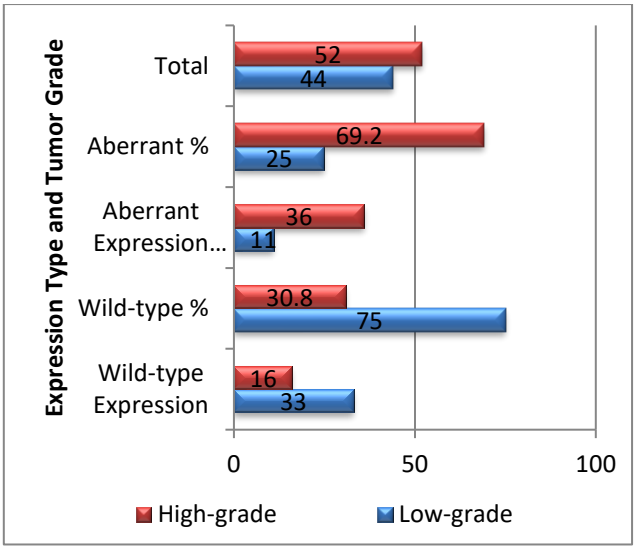

Figure 2. Phenotypic classification of p53 and its association with tumor grade.

Discussion

The results of this study demonstrated a significant association between p53 immunoexpression and the histologic grade of urothelial carcinoma. A greater proportion of high-grade tumors showed p53 positivity, particularly at both 10% and 40% cut-offs, compared to low-grade tumors. The frequency of aberrant p53 phenotypes, including overexpression and null types, was also markedly higher in high-grade cases. Furthermore, p53 expression showed significant correlation with lamina propria and muscularis propria invasion, highlighting its potential value not only as a grading marker but also as an indicator of tumor invasiveness. These findings suggest that p53 is a relevant prognostic marker that may aid in stratifying patients based on tumor behavior and progression.

When compared with existing literature, the results align with global trends in p53 research[19]. Studies have consistently reported that high-grade urothelial carcinomas exhibit elevated p53 immunoexpression, supporting the role of p53 as a marker of poor differentiation and aggressive tumor biology.[20] Similarly, the correlation of p53 overexpression with increased depth of invasion has been well documented, confirming that TP53 mutations are often associated with more invasive phenotypes.[21] The phenotypic classification used in this study (wild-type vs. aberrant) also supports findings from recent literature, which suggest that analyzing aberrant p53 expression patterns (null or diffuse overexpression) provides a more reliable assessment of TP53 mutational status than using percentage cut-offs alone.[22] Additionally, tumors expressing aberrant phenotypes of p53 have been associated with chemoresistance and disease recurrence, emphasizing the need for integrating p53 IHC evaluation in routine diagnostics, particularly in high-grade cases.[23]

Furthermore, the current study supports evidence indicating that p53 immunoreactivity is significantly higher in tumors with lamina propria and muscularis propria invasion.[24] Literature has also documented that TP53 mutations promote epithelial-to-mesenchymal transition, leading to increased tumor cell migration and invasion.[25] Therefore, the presence of diffuse or null p53 staining may serve as a warning sign for early invasive potential, even in cases that appear non-invasive on histology.[26] The data from this study corroborate these observations and reinforce the use of p53 as a cost-effective, practical prognostic marker, especially in settings where advanced molecular testing may not be readily available.[27]

Limitations and Future Recommendations: One limitation of this study was the absence of molecular confirmation of TP53 mutations, which could have validated the immunohistochemical findings at the genetic level. Additionally, the sample size was modest, and the study was conducted at a single center, limiting the generalizability of results. Future research should focus on multi-center studies with larger cohorts and incorporate molecular sequencing to correlate immunohistochemistry findings with underlying genetic mutations. It is also recommended to explore the combined expression profiles of multiple markers, such as p53, Ki-67, and p16, to develop more robust prognostic panels for urothelial carcinoma.

Conclusion

This study demonstrated a significant association between p53 immunoexpression and both tumor grade and depth of invasion in urothelial carcinoma. Tumors with lamina propria or muscularis propria invasion had significantly higher expression of p53 again in high cut-offs and phenotypic classifications. The results allow to suppose that p53 may become a steady prognostic marker, helping to identify more aggressive tumors with a higher invasive potential. The use of p53 immunohistochemistry as part of daily diagnostic assessment could help streamline the process of stratification of risk as well as the development of therapeutic plans, particularly in disadvantaged settings. These findings further corroborate the need to incorporate the use of immunomarkers such as p53 that can add value in the determination of the histologic grading of urothelial carcinoma.

References

1. Murta-Nascimento C, Schmitz-Dräger BJ, Zeegers MP, Steineck G, Kogevinas M, Real FX, Malats N. Epidemiology of urinary bladder cancer: from tumor development to patient's death. World journal of urology. 2007 Jun;25(3):285-95.
2. Parkin DM. Global cancer statistics in the year 2000. The lancet oncology. 2001 Sep 1;2(9):533-43.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018 Nov;68(6):394-424.
4. Ikram A, Pervez S, Khadim MT, Sohaib M, Uddin H, Badar F, Masood AI, Khattak ZI, Naz S, Rahat T, Murad N. National cancer registry of Pakistan: first comprehensive report of cancer statistics 2015-2019. J Coll Physicians Surg Pak. 2023 Jun 1;33(6):625-32.
5. Köbel M, Ronnett BM, Singh N, Soslow RA, Gilks CB, McCluggage WG. Interpretation of P53 immunohistochemistry in endometrial carcinomas: toward increased reproducibility. International journa of gynecological pathology. 2019 Jan 1;38:S123-31..
6. Moch H, Cubilla AL, Humphrey PA, Reuter VE, Ulbright TM. The 2016 WHO classification of tumours of the urinary system and male genital organs—part A: renal, penile, and testicular tumours. European urology. 2016 Jul 1;70(1):93-105.
7. Lopez-Beltran A. Bladder cancer: clinical and pathological profile. Scandinavian journal of urology and nephrology. 2008 Jan 1;42(sup218):95-109.
8. Epstein JI. Diagnosis and reporting of limited adenocarcinoma of the prostate on needle biopsy. Modern pathology. 2004 Mar;17(3):307-15.
9. Soria F, Krabbe LM, Todenhöfer T, Dobruch J, Mitra AP, Inman BA, Gust KM, Lotan Y, Shariat SF. Molecular markers in bladder cancer. World journal of urology. 2019 Jan 23;37(1):31-40.
10. Levine AJ, Finlay CA, Hinds PW. P53 is a tumor suppressor gene. Cell. 2004 Jan 23;116:S67-70.
11. Bates S, Vousden KH. Mechanisms of p53-mediated apoptosis. Cellular and Molecular Life Sciences CMLS. 1999 Jan;55(1):28-37.
12. Ciccarese C, Massari F, Blanca A, Tortora G, Montironi R, Cheng L, Scarpelli M, Raspollini MR, Vau N, Fonseca J, Lopez-Beltran A. Tp53 and its potential therapeutic role as a target in bladder cancer. Expert opinion on therapeutic targets. 2017 Apr 3;21(4):401-14.
13. Esrig D, Elmajian D, Groshen S, Freeman JA, Stein JP, Chen SC, Nichols PW, Skinner DG, Jones PA, Cote RJ. Accumulation of nuclear p53 and tumor progression in bladder cancer. New England Journal of Medicine. 1994 Nov 10;331(19):1259-64.
14. Shariat SF, Lotan Y, Vickers A, Karakiewicz PI, Schmitz-Dräger BJ, Goebell PJ, Malats N. Statistical consideration for clinical biomarker research in bladder cancer. InUrologic Oncology: Seminars and Original Investigations 2010 Jul 1 (Vol. 28, No. 4, pp. 389-400). Elsevier.
15. Shvarts O, Seligson D, Lam J, Shi T, Horvath S, Figlin R, Belldegrun A, Pantuck AJ. p53 is an independent predictor of tumor recurrence and progression after nephrectomy in patients with localized renal cell carcinoma. The Journal of urology. 2005 Mar;173(3):725-8.
16. Abubakar M, Guo C, Koka H, Sung H, Shao N, Guida J, Deng J, Li M, Hu N, Zhou B, Lu N. Clinicopathological and epidemiological significance of breast cancer subtype reclassification based on p53 immunohistochemical expression. NPJ breast cancer. 2019 Jul 25;5(1):20.
17. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer research. 1994 Sep 15;54(18):4855-78.
18. Bukhari MH, Niazi S, Khaleel ME, Sharif MA, Ghani R, Mehmood MT, Tahseen M, Chaudhry NA, Hasan M. Elevated frequency of p53 genetic mutations and AgNOR values in squamous cell carcinoma. Journal of cutaneous pathology. 2009 Feb;36(2):220-8.
19. Parkin DM. The global burden of urinary bladder cancer. Scandinavian journal of urology and nephrology. 2008 Jan 1;42(sup218):12-20.
20. Shariat SF, Tokunaga H, Zhou J, Kim J, Ayala GE, Benedict WF, Lerner SP. p53, p21, pRB, and p16 expression predict clinical outcome in cystectomy with bladder cancer. Journal of clinical oncology. 2004 Mar 15;22(6):1014-24.
21. Ciccarese C, Massari F, Blanca A, Tortora G, Montironi R, Cheng L, Scarpelli M, Raspollini MR, Vau N, Fonseca J, Lopez-Beltran A. Tp53 and its potential therapeutic role as a target in bladder cancer. Expert opinion on therapeutic targets. 2017 Apr 3;21(4):401-14.
22. Lee H, Cho YA, Kim DG, Son JY, Cho EY. Real-world comparison of P53 immunohistochemistry and TP53 mutation analysis using next-generation sequencing. Anticancer research. 2024 Sep 1;44(9):3983-94.
23. Reles A, Wen WH, Schmider A, Gee C, Runnebaum IB, Kilian U, Jones LA, El-Naggar A, Minguillon C, Schönborn I, Reich O. Correlation of p53 mutations with resistance to platinum-based chemotherapy and shortened survival in ovarian cancer. Clinical Cancer Research. 2001 Oct 1;7(10):2984-97.
24. Bernardini S, Billerey C, Martin M, Adessi GL, Wallerand H, Bittard H. The predictive value of muscularis mucosae invasion and p53 over expression on progression of stage T1 bladder carcinoma. The Journal of urology. 2001 Jan 1;165(1):42-6.
25. Muller PA, Vousden KH, Norman JC. p53 and its mutants in tumor cell migration and invasion. Journal of Cell Biology. 2011 Jan 24;192(2):209-18.
26. Marsit CJ, Houseman EA, Schned AR, Karagas MR, Kelsey KT. Promoter hypermethylation is associated with current smoking, age, gender and survival in bladder cancer. Carcinogenesis. 2007 Aug 1;28(8):1745-51.
27. Ahmad HS, Zafer S, Qadri HM, Bashir A. Limitless potential within limited resources: The realm of liquid biopsy for brain tumors in low-middle-income countries. Brain & Spine. 2024 Apr 21;4:102817.