

Epigenetic DNA methylation markers: a non-invasive diagnostic approach for urinary bladder cancer

Amandeep Singh¹, Bhasker Mukherjee², Mukul Sharma³ and Rattan Deep Singh^{1,*}

¹School of Bioengineering and Biosciences, Lovely Professional University, Phagwara 144 411, India

²Department of Biochemistry, Armed Forces Medical College, Pune 411 040, India

³Department of Biochemistry, Pacific Medical College and Hospital, Udaipur 313 024, India

Urinary bladder cancer (UBC) has high recurrence rates, making early detection vital. Promoter DNA methylation of *CALCA*, *CCNA1* and *P16* genes can serve as non-invasive biomarkers. The objective of the present study is to evaluate the promoter methylation status of *CCNA1*, *CALCA* and *P16* genes in the blood of UBC patients compared to controls, and to explore their utility as non-invasive biomarkers. Blood samples from 169 participants, including 89 UBC patients and 80 controls, were analysed. Blood samples were collected, DNA was extracted for bisulphite conversion, followed by methylation-specific polymerase chain reaction (PCR). Statistical analyses determined the association between methylation status and clinical features. Methylation was detected in *CCNA1* (66.2%), *CALCA* (53.9%), and *P16* (72%) in UBC patients, significantly higher than in controls. Combined analysis of these three genes provided a sensitivity of 85% and specificity of 80% for UBC detection. Blood-based methylation of *CCNA1*, *CALCA* and *P16* genes emerged as a promising non-invasive biomarker for early UBC detection. Further validation in larger cohorts is required.

Keywords: DNA methylation, methylation-specific PCR, non-invasive biomarkers, urinary bladder cancer.

origin, whereas around 5% are squamous cell carcinoma³. Despite treatment, including transurethral resection of tumours and adjunctive intravesical therapies, recurrence rates remain high, with 50–70% of patients experiencing tumour recurrence⁴ and 20–30% progressing to advanced stages.

The median age of bladder cancer diagnosis is between 65 and 70 years, with a strong male preponderance. Environmental and lifestyle factors play crucial roles in the development of UBC, with tobacco smoking being a significant risk factor, accounting for about 75% of cases. Occupational exposure to carcinogenic chemicals, particularly among labourers and industrial workers such as those in the leather, textile, and dye industries, is also linked to an increased risk of bladder cancer. The most common presenting symptom is painless haematuria, with other lower urinary tract symptoms occurring in a substantial number of patients.

The current gold standard for UBC diagnosis and monitoring is cystoscopy with biopsy. Cystoscopy provides direct visualisation of the bladder tissue but is invasive and requires lifelong monitoring due to the high recurrence risk. Urine cytology, although non-invasive, has limited sensitivity for detecting low-grade tumours, making it ineffective as a standalone diagnostic tool. As a result, there is an urgent need for non-invasive, reliable biomarkers for early