Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 24 May 2026
Revised 30 June 2026
Accepted 16 July 2026

Natural Resources for Human Health 2026, Vol. 6 (8s): 846–851

KEYWORDS:
Bladder cancer; Non-muscle-invasive bladder cancer; NDRG1; Biomarker; ELISA

Serum NDRG1 Concentrations in Newly Diagnosed Non-Muscle-Invasive Bladder Transitional Cell Carcinoma: A Case-Control Study

Wesam A. Salh[1], Rawaa H. Ali[2], Mohammed B. Ismail[3]

[1,2]Department of Clinical Biochemistry, College of Medicine, University of Baghdad, Baghdad, Iraq.
[3]Department of Surgery, College of Medicine, University of Baghdad, Baghdad, Iraq.
*Corresponding Author Wesam A. Salh/ Department of Biochemistry, College of Medicine, University of
Baghdad, Baghdad, Iraq. Mobile: 009647707976799 Email: wesam.a.salh@baghdadcollege.edu.iq

ABSTRACT:

Background: Non-muscle-invasive bladder cancer (NMIBC) represents a major proportion of newly diagnosed bladder cancer cases and remains associated with a substantial risk of recurrence. N-myc downstream-regulated gene 1 (NDRG1) is an intracellular protein involved in cellular differentiation, stress responses, and signaling pathways related to tumor biology. Although altered NDRG1 expression has been reported in bladder cancer, evidence regarding its circulating serum concentration in newly diagnosed NMIBC remains limited.

Objective: To compare serum NDRG1 concentrations between patients with histopathologically confirmed newly diagnosed non-muscle-invasive bladder transitional cell carcinoma (NMIBC-TCC) and healthy controls.

Materials and Methods: This hospital-based case-control study included 150 participants, comprising 75 patients with histopathologically confirmed newly diagnosed NMIBC-TCC and 75 apparently healthy controls. Serum NDRG1 concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA). Statistical analyses were performed using the independent-samples Student's t-test and the Chi-square test, with $p < 0.05$ considered statistically significant.

Results: Patients with NMIBC-TCC were significantly older than healthy controls and included higher proportions of males and urban residents, whereas smoking status and family history did not differ significantly between the two groups. Mean serum NDRG1 concentration was marginally higher in patients than in controls (0.996 ± 0.127 vs. 0.965 ± 0.143 ng/mL); however, the difference was not statistically significant ($p = 0.158$).

Conclusion: Serum NDRG1 concentrations did not differ significantly between patients with newly diagnosed NMIBC-TCC and healthy controls. Further studies involving larger cohorts, different disease stages, and longitudinal follow-up are warranted to clarify the clinical and prognostic relevance of circulating NDRG1 in bladder cancer.

1. INTRODUCTION

Bladder cancer is a major malignancy of the urinary tract, with urothelial carcinoma representing the predominant histological type. A substantial proportion of patients present with non-muscle-invasive disease, which comprises papillary tumors classified as Ta, tumors invading the lamina propria (T1), and carcinoma in situ (CIS). [1] Although non-muscle-invasive bladder cancer (NMIBC) is confined to the mucosa or lamina propria, its clinical management remains challenging because of the substantial risk of recurrence and the potential for disease progression in a subset of patients. [2] The diagnosis of non-

muscle-invasive bladder transitional cell carcinoma (NMIBC-TCC) relies primarily on cystoscopic assessment and histopathological evaluation of tumor tissue, creating continued interest in molecular and circulating biomarkers that may complement established diagnostic approaches and provide additional biological information in localized disease. [3,4]

N-myc downstream-regulated gene 1 (NDRG1) is a stress-responsive intracellular protein belonging to the NDRG family and is structurally related to the α/β-hydrolase superfamily, although it lacks conventional hydrolase enzymatic activity. [5] NDRG1 participates in diverse cellular processes, including cellular differentiation, growth regulation, stress adaptation, and maintenance of epithelial integrity; its expression is also responsive to cellular conditions such as hypoxia. Its biological functions are associated with signaling pathways involved in proliferation, apoptosis, migration, and invasion, including PI3K/AKT, NF-κB, and WNT/β-catenin signaling. [6] NDRG1 expression is also regulated by transcriptional factors, including c-Myc and N-Myc, further supporting its involvement in tumor-related cellular processes and tumor biology. [7]

The role of NDRG1 in cancer is highly context-dependent and varies according to tumor type, molecular environment, subcellular localization, and associated signaling pathways. In several malignancies, NDRG1 has been associated with suppression of epithelial-to-mesenchymal transition (EMT), reduced cell migration and metastatic potential, whereas increased NDRG1 expression has been linked to tumor progression and pro-oncogenic phenotypes in other cancer types. [8] This biological heterogeneity has also been observed in urothelial bladder carcinoma, where increased NDRG1 expression has been associated with higher tumor grade, advanced stage, lymphovascular invasion, and other aggressive tumor characteristics. [9] Thus, the biological significance of NDRG1 requires interpretation within the specific molecular and cellular context of the disease in which it is evaluated. [10]

Importantly, NDRG1 has been investigated at the cellular and tissue levels, whereas evidence regarding its circulating presence and clinical significance remains limited. As an intracellular protein, its expression and localization within cells may not directly correspond to its circulating concentration, which has been detected in plasma and serum under different pathological conditions. [11,12] Therefore, tissue expression and circulating NDRG1 represent distinct biological measurements and should not be considered interchangeable; alterations in bladder tumor tissue expression should not be assumed to correspond directly to changes in serum NDRG1.

Evidence regarding circulating NDRG1 in bladder cancer remains limited, with an Iraqi case-control study reporting significantly higher serum NDRG1 concentrations in patients with bladder cancer than in healthy controls. [13] However, differences in patient selection, disease spectrum, clinicopathological characteristics, and analytical conditions may influence circulating biomarker concentrations. [14] In this context, the relevance of serum NDRG1 specifically in patients with histopathologically confirmed, newly diagnosed localized NMIBC-TCC remains insufficiently established. Accordingly, the present study investigated serum NDRG1 concentrations in patients with histopathologically confirmed, newly diagnosed localized NMIBC-TCC and healthy controls using a case-control design. The study focused on patients with newly diagnosed localized disease and healthy controls to determine whether serum NDRG1 concentrations differed between the two groups.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This hospital-based case-control study was conducted at the Department of Biochemistry, College of Medicine, University of Baghdad, Iraq, between June 2025 and February 2026. A total of 150 participants were enrolled, including 75 patients with histopathologically confirmed newly diagnosed NMIBC-TCC and 75 apparently healthy controls. Patients were recruited from Ghazi Hariri Hospital for Surgical Specialties, Baghdad, Iraq. The diagnosis of NMIBC-TCC was established by histopathological examination of tissue specimens obtained following transurethral resection of the bladder tumor (TURBT). The study protocol was approved by the Ethics Committee of the College of Medicine, University of Baghdad, and written informed consent was obtained from all participants before enrollment. All study procedures were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

2.2 Eligibility Criteria

Patients were included if they were adults with newly diagnosed, histopathologically confirmed urothelial (transitional cell) carcinoma of the bladder, comprising pathological stages Ta, T1, and CIS. Patients with benign bladder tumors, muscle-invasive or metastatic bladder cancer, other malignancies, acute lower urinary tract infection, or those considered unfit for surgery were excluded. The control group consisted of apparently healthy individuals recruited from the community and

companions of patients attending the study hospital, with no previous history of bladder cancer or other known malignant disease.

2.3 Blood Sample Collection and Serum Preparation

Approximately 6 mL of venous blood was collected aseptically from each participant between 8:30 a.m. and 1:30 p.m. into gel separator tubes. Following complete clot formation, the blood samples were centrifuged at 4000 rpm for 10 minutes. The separated serum was transferred into appropriately labeled Eppendorf tubes and stored at −80°C until biochemical analysis.

2.4 Determination of Serum NDRG1

Serum NDRG1 concentrations were measured using a commercially available Human NDRG1 ELISA kit (Protein NDRG1, FineTest®, Wuhan, China; Catalog No. EH1617), according to the manufacturer's instructions. All serum samples were analyzed under the same laboratory conditions. Absorbance was measured at 450 nm using an ELISA microplate reader, and NDRG1 concentrations were determined from the standard curve. The results were expressed as ng/mL.

2.5 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), whereas categorical variables were presented as frequencies and percentages. Differences between the patient and control groups were assessed using the independent-samples Student's t-test for continuous variables and the Chi-square test for categorical variables. A two-tailed p-value of <0.05 was considered statistically significant.

3. RESULTS

3.1 Participant Characteristics

A total of 150 participants were enrolled in this case-control study, comprising 75 patients with histopathologically confirmed newly diagnosed NMIBC-TCC and 75 healthy controls. This study showed significant differences between the two groups in terms of age, sex, and residence. The NMIBC-TCC group had a significantly older demographic, with 73.3% of patients aged 50 or older ($p = 0.001$). Sex also differs significantly, as males comprise 80% of the NMIBC-TCC group compared to 64% of the controls ($p = 0.029$). Urban living is significantly more prevalent in the NMIBC-TCC group (76%) than in the control group (60%; $p = 0.036$). Conversely, no significant difference was detected between the study groups and smoking status ($p = 0.739$) or family history of bladder cancer ($p = 0.821$). The demographic characteristics of the study participants are presented in Table 1.

Table 1. Demographic Characteristics of Study Participants

Variable	Study groups		p-value
	NMIBC-TCC group (%) n= 75	Control group (%) n= 75	
Age (years)			
< 50	20 (26.7)	41 (54.7)	**0.001**
≥ 50	55 (73.3)	34 (45.3)	
Gender			
Male	60 (80.0)	48 (64.0)	**0.029**
Female	15 (20.0)	27 (36.0)	
Residence			
Rural	18 (24.0)	30 (40.0)	**0.036**

Urban	57 (76.0)	45 (60.0)	
Smoking			
Smoker	31 (41.3)	29 (38.7)	**0.739**
Nonsmoker	44 (58.7)	46 (61.3)	
Family history of bladder cancer			
Yes	12 (16.0)	11 (14.7)	**0.821**
No	63 (84.0)	64 (85.3)	

* Significant difference between percentages using Pearson Chi-square test at 0.05 level.

3.2 Serum NDRG1 Concentration

Serum NDRG1 concentrations were evaluated in patients with NMIBC-TCC and healthy controls. The mean serum NDRG1 concentration was marginally higher in patients with NMIBC-TCC than in healthy controls (0.996 ± 0.127 vs. 0.965 ± 0.143 ng/mL); however, the difference was not statistically significant (p = 0.158). The results are summarized in Table 2.

Table 2. Comparison of Serum NDRG1 Concentrations

Variable	**Study groups**		**p-value**
	NMIBC-TCC group (n= 75) Mean ± SD	**Control group (n=75) Mean ± SD**	
NDRG1 (ng/mL)	0.996 ± 0.127	0.965 ± 0.143	**0.158**

* Significant difference between two independent means using Student's t-test at the 0.05 level.

4. DISCUSSION

The present study demonstrated no statistically significant difference in serum NDRG1 levels between patients with histopathologically confirmed localized NMIBC-TCC and healthy controls (0.996 ± 0.127 vs. 0.965 ± 0.143 ng/mL, p = 0.158). This finding suggests that serum NDRG1 may have limited utility for distinguishing patients with localized NMIBC-TCC from healthy individuals. NDRG1 is an intracellular protein involved in cellular differentiation, proliferation, stress responses, and multiple signaling pathways associated with tumor biology. [15] Alterations in tissue expression may not necessarily correspond to changes in circulating NDRG1 concentrations, as tissue and circulating measurements represent distinct biological compartments. [12] These findings suggest that circulating NDRG1 may not adequately reflect tissue-specific molecular alterations in localized NMIBC-TCC.

Previous investigations of NDRG1 in bladder cancer have included tissue-based studies, whereas evidence regarding serum NDRG1 remains limited. Tissue-based studies have demonstrated altered NDRG1 expression and have linked its upregulation to EMT, tumor progression, and poor clinical outcome. [16] NDRG1 has also been evaluated in urine as a noninvasive biomarker of bladder cancer, with urinary NDRG1 levels showing associations with pathological stage and tumor grade. [17] Direct comparison between the present serum-based findings and previous tissue- and urine-based reports should be interpreted with caution because biological specimens provide distinct information. Accordingly, serum, urine, and tissue NDRG1 assessments should be considered complementary rather than directly comparable.

The biological significance of NDRG1 appears to be highly dependent on tumor type, molecular characteristics, and disease stage. [10] Previous evidence has shown that NDRG1 may function either as a tumor suppressor or as a tumor promoter depending on the cellular context and the signaling pathways involved. [18] This biological heterogeneity may account for the inconsistent patterns of NDRG1 expression reported across different malignancies. The absence of a significant difference in circulating NDRG1 observed in the present study should be interpreted within this biological context rather than as evidence against its involvement in bladder carcinogenesis.

Unlike the present findings, an Iraqi case-control study reported significantly higher serum NDRG1 levels in patients with bladder cancer than in healthy controls, suggesting a potential diagnostic role for this biomarker. [13] The discrepancy between the two studies may be explained by differences in patient selection, disease stage, clinicopathological characteristics, sample size, and analytical methodology. In addition, the present study was restricted to patients with histopathologically confirmed localized NMIBC-TCC, whereas variation in disease spectrum may substantially influence circulating biomarker concentrations. These methodological and clinical differences provide a plausible explanation for the contrasting findings between the two studies.

From a diagnostic perspective, the present findings suggest that serum NDRG1 alone may have limited utility for distinguishing patients with localized NMIBC-TCC from healthy individuals. Nevertheless, this observation does not exclude the biological relevance of NDRG1 in bladder cancer, as its diagnostic utility may depend on the biological specimen analyzed and the context in which it is evaluated. Recent advances in bladder cancer research support the integration of multiple biomarkers rather than reliance on a single molecular marker for diagnostic decision-making. [19] Serum NDRG1 may therefore have greater value when interpreted alongside other established biomarkers.

Overall, the present findings suggest that serum NDRG1 may not adequately reflect tissue-specific molecular alterations in localized NMIBC-TCC. Future studies evaluating molecular markers across different disease stages and biological compartments may help clarify their clinical relevance. [20] Combining serum and tissue NDRG1 assessment in larger cohorts may help determine whether circulating NDRG1 has a potential role within multimarker diagnostic strategies rather than as a stand-alone biomarker.

5. LIMITATIONS

Several limitations should be considered when interpreting these findings. The relatively modest sample size and single-center case-control design may limit the generalizability of the results and the ability to detect smaller differences in serum NDRG1 concentrations. In addition, NDRG1 is primarily an intracellular protein, and its serum concentration may not directly reflect tissue-specific molecular alterations. Differences in demographic characteristics between the study groups may also have introduced potential confounding that was not fully addressed in the present analysis. Finally, serum NDRG1 was assessed at a single time point at initial diagnosis, precluding evaluation of longitudinal changes or prognostic outcomes such as recurrence and disease progression. Further multicenter studies with larger cohorts and longitudinal follow-up are warranted to clarify the clinical significance of circulating NDRG1 in NMIBC.

6. CONCLUSION

Serum NDRG1 concentrations did not differ significantly between patients with histopathologically confirmed newly diagnosed NMIBC-TCC and healthy controls. Further studies involving larger cohorts, different disease stages, and longitudinal follow-up are warranted to determine whether circulating NDRG1 has clinical or prognostic relevance in NMIBC.

REFERENCES

[1] Mancini M, Righetto M, Noessner E. Checkpoint Inhibition in Bladder Cancer: Clinical Expectations, Current Evidence, and Proposal of Future Strategies Based on a Tumor-Specific Immunobiological Approach. Cancers (Basel). 2021;13(23):6016. doi:10.3390/cancers13236016.

[2] Miyake M, Nishimura N, Fujii T, Fujimoto K. Recent advancements in the diagnosis and treatment of non-muscle invasive bladder cancer: Evidence update of surgical concept, risk stratification, and BCG-treated disease. Int J Urol. 2023;30(11):944-957. doi:10.1111/iju.15263.

[3] Zhao J, Li J, Zhang R. Off the fog to find the optimal choice: Research advances in biomarkers for early diagnosis and recurrence monitoring of bladder cancer. Biochim Biophys Acta Rev Cancer. 2023;1878(4):188926. doi:10.1016/j.bbcan.2023.188926.

[4] Kalaf FH, Rasheed MK, Ismail MB. Assessment of urine and serum exosomes as biomarkers for the diagnosis of bladder cancer. Al-Rafidain J Med Sci. 2024;6(2):101-105. doi:10.54133/ajms.v6i2.758.

[5] Mustonen V, Muruganandam G, Loris R, Kursula P, Ruskamo S. Crystal and solution structure of NDRG1, a membrane-binding protein linked to myelination and tumour suppression. FEBS J. 2021;288(11):3507-3529. doi:10.1111/febs.15660.

[6] Chekmarev J, Gholam Azad M, Richardson DR. The oncogenic signaling disruptor, NDRG1: molecular and cellular mechanisms of activity. Cells. 2021;10(9):2382. doi:10.3390/cells10092382.
[7] Deng Z, Richardson DR. The Myc family and the metastasis suppressor NDRG1: targeting key molecular interactions with innovative therapeutics. Pharmacol Rev. 2023;75(5):1007-1035. doi:10.1124/pharmrev.122.000795.
[8] Park KC, Paluncic J, Kovacevic Z, Richardson DR. Pharmacological targeting and the diverse functions of the metastasis suppressor, NDRG1, in cancer. Free Radic Biol Med. 2020;157:154-175. doi:10.1016/j.freeradbiomed.2019.05.020.
[9] Louis EK, Abdelkawi IF, Refaiy A, Ahmed AM. N-myc downstream-regulated gene 1 can promote vasculogenic mimicry and angiogenesis in urothelial carcinoma. Virchows Arch. 2024;484(5):827-836. doi:10.1007/s00428-024-03793-w.
[10] Ghafouri-Fard S, Ahmadi Teshnizi S, Hussen BM, Taheri M, Sharifi G. A review on the role of NDRG1 in different cancers. Mol Biol Rep. 2023;50(7):6251-6264. doi:10.1007/s11033-023-08540-z.
[11] Li S, Xin K, Pan S, Wang Y, Zheng J, Li Z, et al. Blood-based liquid biopsy: insights into early detection, prediction, and treatment monitoring of bladder cancer. Cell Mol Biol Lett. 2023;28:28. doi:10.1186/s11658-023-00442-z.
[12] Saponaro C, Gammaldi N, Cavallo V, Ramírez-Morales MA, Zito FA, Sonnessa M, et al. Insight into the regulation of NDRG1 expression. Int J Mol Sci. 2025;26(8):3582. doi:10.3390/ijms26083582.
[13] Ibraheem NIA, Ali RH, Ismail MB. NDRG1 is being investigated as a possible bladder cancer biomarker in the Iraqi population. Rev Bionatura. 2022;7(4):23. doi:10.21931/RB/2022.07.04.23.
[14] Hensley PJ, Teoh JYC, Li R, et al. Integrating clinically actionable biomarkers into bladder cancer care — recommendations from the International Bladder Cancer Group. Nat Rev Urol. 2026. doi:10.1038/s41585-026-01179-y.
[15] Gholam Azad M, Russell TM, Gu X, Zhao X, Richardson V, Wijesinghe TP, et al. NDRG1 and its family members: more than just metastasis suppressor proteins and targets of thiosemicarbazones. J Biol Chem. 2025;301(7):110230. doi:10.1016/j.jbc.2025.110230.
[16] Li A, Zhu X, Wang C, Yang S, Qiao Y, Qiao R, et al. Upregulation of NDRG1 predicts poor outcome and facilitates disease progression by influencing the EMT process in bladder cancer. Sci Rep. 2019;9(1):5166. doi:10.1038/s41598-019-41660-w.
[17] Sun YJ, Li AW, Zhang YC, Dai JH, Zhu X. Evaluation of urine NDRG1 as noninvasive biomarker for bladder cancer diagnosis. Clin Lab. 2021;67(3). doi:10.7754/Clin.Lab.2020.191020.
[18] Joshi V, Lakhani SR, McCart Reed AE. NDRG1 in cancer: a suppressor, promoter, or both? Cancers (Basel). 2022;14(23):5739. doi:10.3390/cancers14235739.
[19] Ahangar M, Mahjoubi F, Mowla SJ. Bladder cancer biomarkers: current approaches and future directions. Front Oncol. 2024;14:1453278. doi:10.3389/fonc.2024.1453278.
[20] Soria F, Krabbe LM, Todenhöfer T, Dobruch J, Mitra AP, Inman BA, et al. Molecular markers in bladder cancer. World J Urol. 2019;37(1):31-40. doi:10.1007/s00345-018-2503-4.