العلاقة بين المستويات البلازمية والنسيجية لبروتين HMGA2 لدى مرضى سرطان الظهارة البولية

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ملخّص

الخلفية: يُشكّل سرطان المثانة، الذي يحتل المرتبة التاسعة عالمياً من حيث الإصابة، تحدياتٍ كبيرةً نظراً لارتفاع معدلات نكسه والوفيات الناجمة عنه. هدفت هذه الدراسة إلى تحري العلاقة بين مستويات بروتين المجموعة عالية الحركة A2 (HMGA2) في البلازما ومستوياته في الأنسجة لدى مرضى سرطان مثانة سوريين مصابين بسرطان الظهارة البولية transitional cell carcinoma (TCC).

المواد والطرائق: أُجريت دراسة مقطعية على 32 مريضاً، وقيست مستويات بروتين HMGA2 في البلازما باستخدام طريقة ELISA، وتم التّحري عن البروتين في النّسيج بالاعتماد على تقانة التلوين المناعي الكيميائي للنسيج IHC.

النتائج: كشفت هذه الدراسة عن وجود ارتباط إيجابي معتدل بين مستويات بروتين HMGA2 في البلازما ومستوياته في الأنسجة (r=0.471، P=0.007).

الاستنتاجات: أظهرت النتائج وجود علاقة ذات دلالة إحصائية بين مستويات بروتين HMGA2 في البلازما والأنسجة، مما يشير إلى إمكانية الاعتماد على قياس مستوى HMGA2 في البلازما كمؤشر حيوي غير باضع لتشخيص وتدبير سرطان الظهارة البولية ، وتؤكد هذه النتائج على أهمية إجراء المزيد من الأبحاث للتحقق للتأكيد على هذه النتائج لدى مجموعات سكانية متنوعة وأكبر.

الكلمات المفتاحية: سرطان الظهارة البولية،HMGA2، ELISA، IHC، بلازما، نسيج .

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Correlation of tissue and plasma levels of HMGA2 protein in transitional cell carcinoma patients

Abstract

- **Background:** Bladder cancer, which ranks ninth globally in terms of incidence, poses significant challenges because of its high recurrence and mortality rates. This study aimed to investigate the correlation between plasma and tissue levels of high mobility group A2 (HMGA2) protein in Syrian bladder cancer patients with transitional cell carcinoma.
- **Materials and methods:** A cross-sectional study was conducted with 32 patients, and HMGA2 protein levels were analyzed via enzyme-linked immunosorbent assay (ELISA) for plasma and immunohistochemistry (IHC) for tissue.
- **Results:** This study revealed a moderate positive correlation between the plasma and tissue levels of the HMGA2 protein ($r=0.471$, $P=0.007$).
- **Conclusions:** The results revealed a statistically significant correlation between plasma and tissue HMGA2 levels, suggesting the potential of plasma HMGA2 level as a noninvasive biomarker for transitional cell carcinoma (in the bladder) diagnosis and management. These findings underscore the importance of further research to validate HMGA2 protein plasma and tissue levels in larger cohorts and diverse populations.

Keywords: TCC, HMGA2, ELISA, IHC, Plasma, Tissue.

Introduction

Bladder cancer (BC) ranks as the ninth most common cancer globally and affects both men and women at a ratio of 4:1. It is characterized by a high risk of recurrence and mortality (1). In Syria, World Health Organization statistics for 2022 reported 721 new cases and 396 deaths of bladder cancer. Early diagnosis is crucial, as prompt detection and treatment significantly increase patient survival rates (2).

Current diagnostic methods rely on cystoscopy following the detection of hematuria, which remains the gold standard for confirming the presence of a tumor. However, cystoscopy is invasive, uncomfortable for patients, and costly (3) (4). Therefore, the adoption of biomarkers that support transitional cell carcinoma (in the bladder) diagnosis and can be measured in a noninvasive manner is essential. High mobility group (HMG) proteins, discovered in 1973 (5), have been proposed as potential biomarkers. These proteins play roles in various stages of carcinogenesis, including the cell cycle, apoptosis, angiogenesis, epithelial–mesenchymal transformation, cancer stem cell promotion, and DNA repair mechanisms. These processes enable cancer cells to survive, proliferate, migrate, and progress. HMGA2, a member of the HMG family and the HMGA subfamily, binds to specific DNA or chromatin structures independently of nucleotide sequence through AT hooks (6) (7). This binding leads to changes in chromatin structure and gene expression regulation (8). Several studies have reported high levels of HMGA2 expression in tissue

samples from various cancers, including breast (9), ovarian (10), and bladder cancers (11) (12). This study aimed to investigate the plasma and tissue levels of the HMGA2 protein and determine the correlation between these levels in Syrian bladder cancer patients with urothelial carcinoma more specifically transitional cell carcinoma (TTC), as previous studies have not explored this topic.

Materials and methods

This cross-sectional study included 32 Syrian patients with TTC. The inclusion criteria were newly diagnosed transitional cell carcinoma patients with no history of other tumors and no prior treatment. On the other hand, this study excluded patients with necrotizing injuries or benign tumors of any type.

Samples and data collection.

Blood samples were collected from patients visiting the Urology Department at The National University Hospital in Damascus between February 2023 and August 2023. Five milliliters of venous blood were drawn from each patient into EDTA-anticoagulant tubes. The plasma was separated and stored at -80°C until analysis. Tissue samples were obtained immediately after surgical removal, preserved in paraffin wax at the pathology laboratory, and later sectioned to measure tissue HMGA2 protein levels.

Patient data were obtained through personal interviews and review of medical files and pathological results. This study received ethical

approval from the Biomedical Research Ethics Board at Damascus University (January 29, 2022; No. 5). Written informed consent was obtained from all participants.

Measuring plasma and tissue HMGA2 protein levels.

The plasma HMGA2 protein concentration was measured via a sandwich enzyme-linked immunosorbent assay (ELISA) via a commercially available Human HMGA2 ELISA kit (ELK Biotechnology, China), whereas tissue HMGA2 protein levels were measured via immunohistochemistry (IHC) via a commercially available APC/CY7-linked polyclonal antibody to high mobility group AT hook protein 2 (HMGA2) kit (MyBioSource, USA).

Immunohistochemical staining evaluation.

Immunohistochemical staining was evaluated by two pathologists blinded to the clinicopathological data. Protein expression was assessed by scanning whole tissue specimens at low magnification (×40) and confirmed at higher magnifications (×100 and ×400). The staining intensity was scored as 0 (no staining), 1 (weakly stained), 2 (moderately stained), or 3 (strongly stained). The extent of staining was scored as 0 (negative), 1 (<10% of the tumor area stained), 2 (10–50% of the tumor area stained), or 3 (>50% of the tumor area stained). The overall score was determined by combining the mean staining intensity and distribution. Scores of 0 and 1 were considered low expression, whereas scores equal to or greater than 2 were considered high expression (Table 1).

Statistical analysis.

Statistical analysis was performed via SPSS version 25 (SPSS Inc., Chicago, IL, USA). The values are presented as the mean ± standard deviations (Stds). The t-test was used to compare values between two groups, whereas Fisher's exact test was used to evaluated relationships between qualitative variables. Spearman's test was used to assess correlations. A P value of < 0.05 was considered statistically significant.

Results

This study included 32 Syrian bladder cancer patients with transitional cell carcinoma.

HMGA2 protein levels in bladder cancer tissue samples.

Immunohistochemical staining revealed that 71.9% (23/32) of the transitional cell carcinoma tissue samples presented high levels of the HMGA2 protein (Table 1).

Table 1. Immunohistochemical staining results of bladder cancer tissue samples and their and the corresponding protein concentrations in plasma.

Parameter	Score	Cases No. (%)	HMGA2 plasma concentrations (ng/ml) (Mean ± Std.)
Staining intensity	0	3 (9.4%)	8.68 ± 1.22
	1	6 (18.8%)	11.72 ± 3.97
	2	18 (56.2%)	14.52 ± 4.05
	3	5 (15.6%)	16.65 ± 4.65
HMGA2 tissue level	Low	9 (28.1%)	10.96 ± 3.67
	High	23 (71.9%)	14.98 ± 4.18

Brown staining was predominantly observed in the nuclei and cytoplasm of the cells (Figure 1).

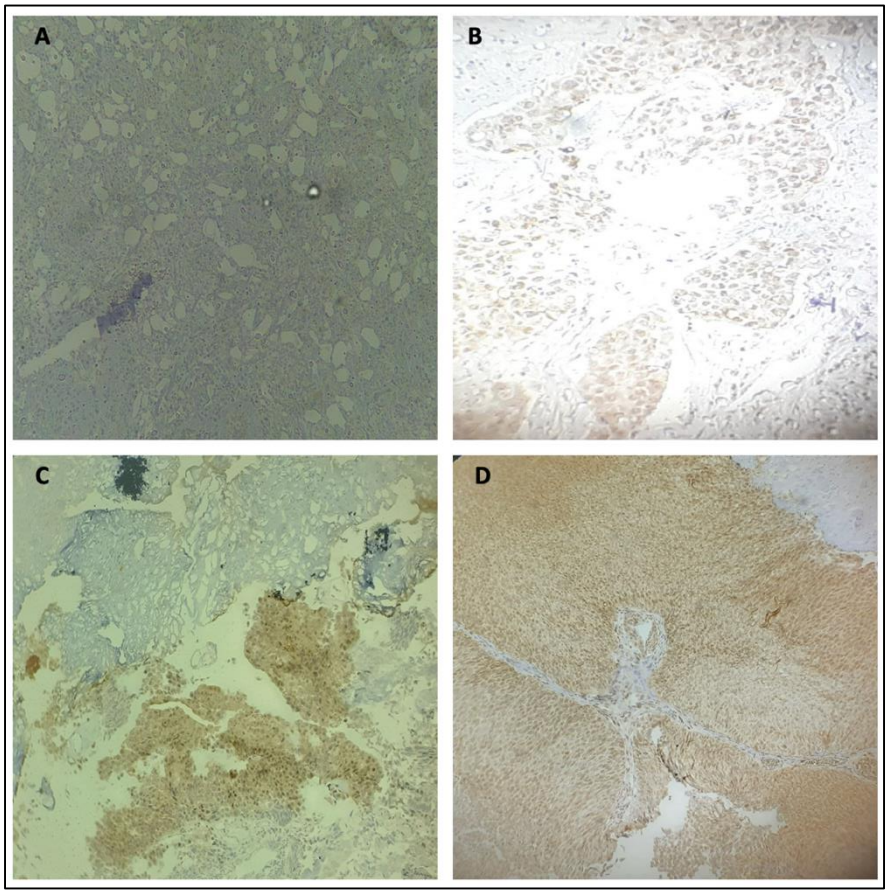

Fig. 1. Immunohistochemical staining of the HMGA2 protein in bladder tissues. (A) Negative HMGA2 staining, (B) weak HMGA2 staining, (C) moderate HMGA2 staining, (D) strong HMGA2 staining

Relationship between plasma and tissue HMGA2 levels.

Spearman's test was applied to study the correlation between plasma HMGA2 levels and tissue levels in patients with transitional cell carcinoma, and a statistically significant correlation was detected (Figure 2), as shown in Table 2.

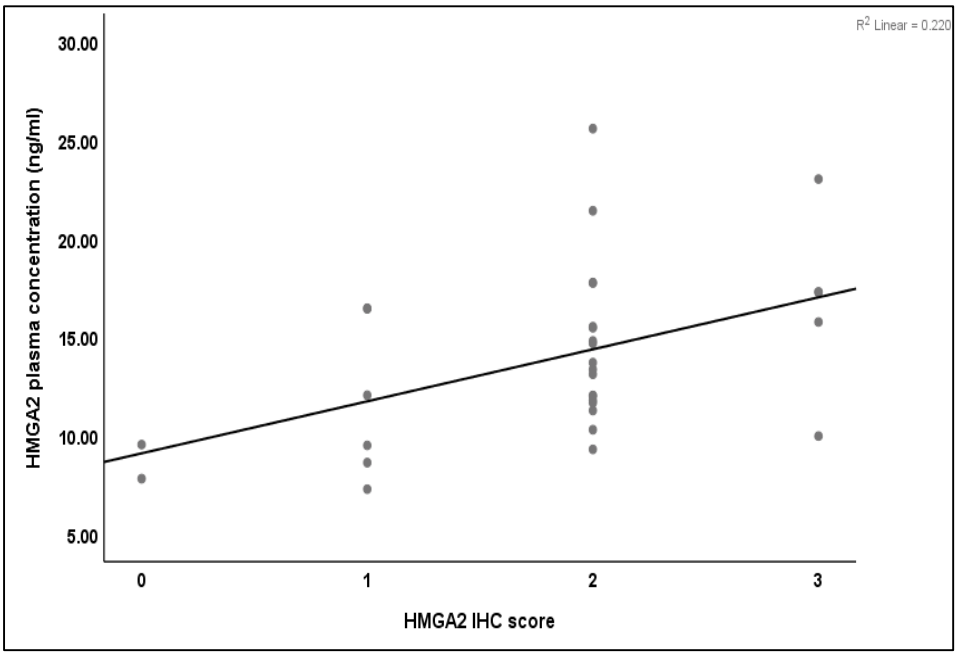

Fig. 2. Correlation between tissue and plasma HMGA2 levels

Table 2. Correlation between plasma and tissue HMGA2 levels.

	HMGA2 plasma levels (ng/ml)	
HMGA2 tissue levels	**Correlation coefficient ***	**P-value ****
	0.471	**0.007**

*** The correlation coefficient was calculated via Spearman correlation analysis.**

**** P-value > 0.05 is not significant.**

Discussion

Late diagnosis of transitional cell carcinoma of the bladder cancer often leads to the need for cystectomy, significantly affecting patients' quality of life. The current diagnostic procedures for transitional cell carcinoma are invasive, painful, and financially burdensome, with associated complications. A noninvasive, easy-to-apply marker capable of detecting transitional cell carcinoma and assisting in disease management has yet to be approved.

This study investigated the correlation between plasma HMGA2 protein levels and tissue levels to determine the potential of using plasma levels as a proxy for tissue levels. Previous studies have shown an association between tissue HMGA2 protein levels and the characteristics of transitional cell carcinoma of the bladder and its poor prognosis. Measuring plasma levels is a simple, painless, noninvasive procedure that could increase patient compliance with diagnostic protocols, especially since the HMGA2 protein has been

detected in some body fluids (13) (14). Some histological studies aimed to investigate the relationships between HMGA2 protein expression levels and the histological and clinical characteristics of patients with bladder cancer and compared them with those in adjacent normal tissues (11) (12) and demonstrated increased expression in transitional cell carcinoma tissues compared with adjacent normal tissues and an association between elevated HMGA2 protein levels and increased tumor stage and grade.

This study revealed a moderate positive correlation between the plasma and tissue levels of the HMGA2 protein. The increased release of the HMGA2 protein into the plasma corresponds with its increased tissue levels. The secretion pathway, which involves extracellular vesicles and caspase-1 mediated pyroptosis, is ER/Golgi-independent (15). Consequently, as tissue HMGA2 expression increases, secretion into the plasma increase. In cancer, the mechanisms regulating HMGA2 protein expression are often disrupted by mutations or deletions in the 3' untranslated region (UTR) of HMGA2 mRNA, which contains miRNA response elements (MREs). This deregulation leads to increased HMGA2 protein expression, influencing various biological processes involved in cancer development and progression (16) (17). Studies indicate that the HMGA2 protein activates the cell cycle and inhibits apoptosis (18) (19). Additionally, the HMGA2 protein plays a significant role in angiogenesis, facilitating cancer invasion of distant organs (20) (21). The HMGA2 protein also contributes to the

epithelial–mesenchymal transformation of cancer cells, increasing their metastatic potential (22). It also stimulates various signal transduction pathways, and reduces cancer cell differentiation (23) (24) (25).

On the bases of these results, plasma HMGA2 levels can be proposed as a biomarker reflecting the histological status of the HMGA2 protein in transitional cell carcinoma tissue samples and as noninvasive indicators contributing to the diagnosis and management of this cancer. This study had several limitations, including a relatively small patient cohort and a single-center design.

Conclusions

This study revealed a significant correlation between plasma and tissue HMGA2 protein levels in Syrian bladder cancer patients with transitional cell carcinoma. These results suggest that plasma HMGA2 levels can serve as a noninvasive biomarker, reflecting the histological HMGA2 protein status in transitional cell carcinoma tissue samples. The ease of measuring plasma HMGA2 levels offers a promising approach for enhancing patient compliance with diagnostic procedures, thereby improving early detection and disease management. However, the study's limitations, including the small patient cohort and single-center design, highlight the need for further research in larger, multicenter studies to validate these findings and

establish HMGA2 as a reliable biomarker for transitional cell carcinoma in the bladder.

Recommendations

This study recommends conducting similar studies on a larger number of patients and including multiple centers to investigate the possibility of proposing the plasma concentration of HMGA2 protein as a potential analysis in the diagnosis approach of transitional cell carcinoma (in the bladder).

Abbreviations

BC: Bladder Cancer, **TTC:** Transitional Cell Carcinoma, **ELISA:** Enzyme-Linked ImmunoSorbent Assay, **HMG:** High Mobility Group, **IHC:** ImmunoHistoChemistry, **MRE:** MicroRNA Response Element, **UTR:** UnTranslated Region.

Declarations

Ethics approval and consent to participate.

Plasma and tissue samples were obtained after ethical approval was obtained from the Biomedical Research Ethics Committee (BMREC) at Damascus University (2022/1/29) (No. 5) in accordance with the Declaration of Helsinki (1964). Written informed consent was obtained from each participant.

Consent for publication.

Not applicable

Availability of data and materials.

All materials and all data generated during this study are included in this article.

Competing interests.

The authors declare that they have no competing interests.

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