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COMPARISON BETWEEN CYTOLOGY & SOME BIOMARKERS FOR DETECTION BLADDER CANCER OF IRAQI PATIENTS

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ABSTRACT

Background: Bladder cancer (BC) is the 4th most frequently occurring malignancy and the 9th most common cause of death worldwide in men. The invasive and metastatic form of the cancer is the main cause of death or unfavorable prognosis for BC patients. The presence of a bladder tumor is often discovered after episodes of painless macroscopic hematuria. At initial diagnosis, the disease is non-muscle-invasive in approximately 75% of patients. In recent years, detecting aberrantly biomarkers in serum has helped in the detection noninvasive of BC. **Objective:** To detect bladder tumors in Iraqi patients. Methods: In this cross-sectional study, sixty cases were suspected of having bladder tumors according to the opinion of the physician and some signs and symptoms, the best sign was hematuria in urine (per diagnosis). An additional 30 healthy controls with similar age and sex were recruited as control groups, with an average age of 37_85 years. from Baghdad Medical City / Ghazi al–Hariri Teaching Hospital and AlKindi Specialized Hospital during the period from September / 2023 to April / 2024. Using enzyme-linked immunosorbent assay compare the results with cytology. **Results:** Out of 60 bladder inflammations, the results of ELISA / BTA were highly significant differences ($p < 0.01$), and ELISA / NMP-22 were nonsignificant differences ($P = 0.092$). In addition. **Conclusions:** cytology needs some confirmative tests to detect bladder cancer.

KEYWORDS: bladder tumors, cytology, ELISA markers.

INTRODUCTION

Bladder cancer (BC) is the 4th most frequently occurring malignancy and the 9th most common cause of death worldwide in men (Sung et al., 2021). The invasive and metastatic form of cancer is the main cause of death or unfavorable prognosis for BC patients (Chen et al., 2015). The presence of a bladder tumor is often discovered after episodes of painless macroscopic hematuria. At initial diagnosis, the disease is non-muscle-invasive in approximately 75% of patients. Since non-muscle invasive bladder cancer (NMIBC) tends to recur or progress to muscle-invasive disease, regular and long-term cystoscope evaluations are mandatory (Degerge et al., 2017). Yet, Urine cytology is used as a complementary and noninvasive tool in patients with high-grade (HG) urothelial bladder tumors, but sensitivity is limited in low-grade (LG) disease (Yafi et al., 2015, Degerge et al., 2017). Numerous studies are being developed to enlarge the diagnostic accuracy of urinary tests and the creation of alternatives

to cytology and/or cystoscopy. A vast quantity of potential biomarkers is described in the literature aiming to detect genomic, transcriptomic, epigenetic, or protein changes in serum or urine samples (Tabayoyong and Kamat, 2018). Nuclear matrix protein 22 (NMP22) is a nuclear matrix protein involved in the distribution of chromatin during mitosis (Al-Delaimy et al., 2022). It is generally elevated in BC but can be present in normal urothelial cells, BTA tests detect human complement factor-H-related protein in the urine which is produced by our bodies to protect cells from complement activation. It has an almost identical structure to the complement factor-H-related protein produced by bladder cancer cells (Maas et al., 2023).

METHODS

Study design and setting

This is a face-to-face interview-based cross-sectional study conducted in Baghdad, Iraq, from September / 2023 to April / 2024. Sixty bladder tumor patients were recruited for the study.

Inclusion and exclusion criteria

The study included sixty cases suspected of having bladder tumors according to the opinion of the physician and some signs and symptoms, in Baghdad, Iraq. Any patient with Renal failure and cancer, also every patient who has pro–diagnosis bladder tumors and prostate cancer has been excluded from our study.

Data collection and outcome measurements

The interview was conducted by the same researcher among participants, with clarification of any question that seemed unclear to make the answer more accurate.

Ethical consideration

The Medical Research Ethics Committee at the College of Health and Medical Technology obtained ethical approval. We solely used the data for this study. We obtained written informed consent from the participants.

Statistical analysis

Statistical analysis was performed by using the SPSS Statistical package (Version 26; SPSS, IBM) and Microsoft Office Excel (2010) for drawing the figures except for the receiver operating characteristic (ROC) curve. Independent samples of students (t-test) were performed for comparisons of quantitative variables between studied groups (Age / Year, ELISA tests). Pearson correlation test detected the relationships between immunological assays & Age / Year. The validity of cytology and ELISA tests was estimated with a ROC curve, cut-off value, area under curve (AUC), sensitivity (%), specificity (%), positive predictive value % (NPV), negative predictive value % (NPV), and accuracy. The statistical significance threshold (P – value) was accepted at $P<0.05$ & $P<0.01$.

RESULTS AND DISCUSSION

Sixty cases were suspected of having bladder tumors according to the opinion of the physician and some signs and symptoms, the best sign was hematuria in urine (per-diagnosis). An additional 30 healthy controls with similar age and sex were recruited as control groups. Table and Figure (1) study the comparison between the patients & control health according to age, sex, and smoking. Show results of

both parameters (gender & age) were non-significant (P> 0.05), which agrees with the plain of our paper which studies the age and gender similarities between control and patients to get high accuracy in the results, but in the smoking table & figure give also non-significant (P = 0.729) that come back for most Iraqi peoples are smokers, this agrees with (Jasim Mohammed et al., 2016). But when compare between the age & gender of patients shows there differ between age groups the most affected (30 – 50) were (38.3%), while (51 – 70) were (36.7%) & (71 – 90) were (25%), the results clear most patients (75%) were between the age of (30 – 70), and the disease is four times more common in men than women the percentage were (81.7%) & (18.3%) respectively, this significant gender difference could point to a variety of underlying factors, including genetic predispositions, lifestyle differences, occupational exposures, or differences in health behaviors between men and women. Also, smoker status where the strongest risk factor for bladder cancer is tobacco smoking, which accounts for (76.7%) of all cases, this high occurrence recommends a strong correlation between smoking and the condition being studied. Smoking is a significant risk factor, contributing to both the development and progression of the disease. That agrees with many world studies (van Hoogstraten et al., 2023, Saginala et al., 2020, Lenis et al., 2020, Catto et al., 2021).

Table (1) *(Distribution of demographics & smoking according to studied groups.)*

Parameters			Studied groups		P – Value
			Control N = 30	Patient N = 60	
Age groups / Year	30 – 50	N	13	23	P = 0.441 NS
		%	43.3%	38.3%	
	51 – 70	N	13	22	
		%	43.3%	36.7%	
	71 – 90	N	4	15	
		%	13.3%	25%	
Sex	Male	N	24	49	P = 0.849 NS
		%	80%	81.7%	
	Female	N	6	11	
		%	20%	18.3%	
Smoking	Smoker	N	22	46	P = 0.729 NS
		%	73.3%	76.7%	
	Non-smoker	N	8	14	
		%	26.7%	23.3%	

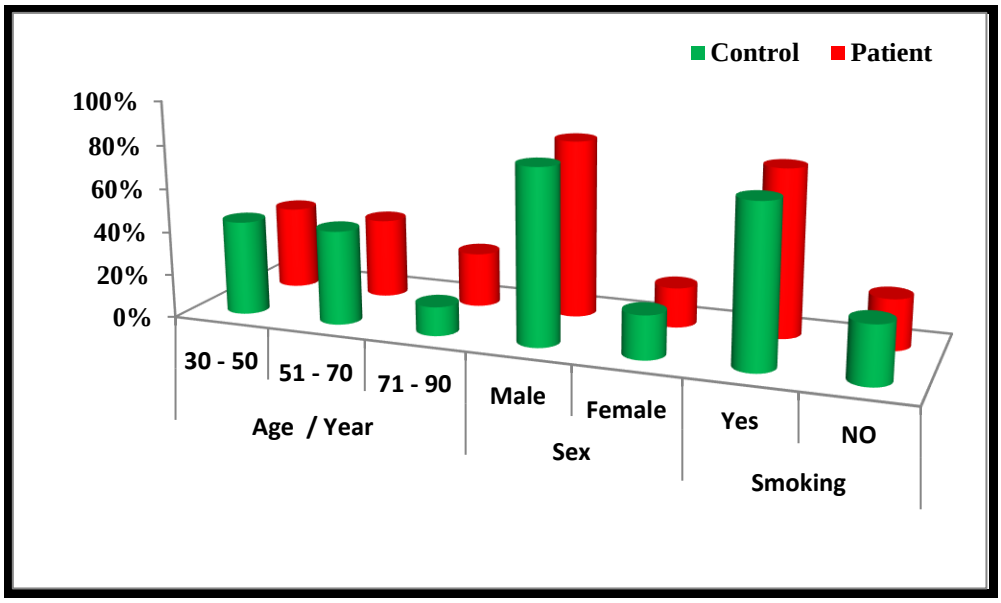

Table (2) revealed significant and highly significant differences between the control and patient groups for several parameters, including chronic bladder inflammation, pioglitazone use in diabetes mellitus, Lynch syndrome, and other genetic syndromes. Chemical substances may affect the infection or alter genetics expression when direct exposure, Certain industries, such as dye, rubber, leather, textiles, and paint, involve exposure to carcinogenic chemicals like aromatic amines, which are linked to an increased risk of bladder cancer, but our study revealed non- significant differ between controls and patients. While the percentage of individuals

exposed to chemicals in the patient group (13.3%) is higher compared to the control group (6.7%), the p-value (0.343) shows that the difference is not statistically significant. This suggests that most of the selected samples were not exposed to chemicals, or the sample size was too small to show a significant change. Chronic bladder problems include a wide range of conditions that affect bladder function & health of the urinary system, Interstitial Cystitis, Overactive Bladder, Chronic Bacterial Cystitis, Radiation Cystitis, Urinary Incontinence, Chemical Cystitis & Neurogenic Bladder. The percentage between patients and controls shows a significant difference (P = 0.017), with prevalence in control groups at 33.3% & patient groups at 60% indicating that chronic bladder problems are a risk factor or a consequence of the condition being studied in the patient group. This agrees with other studies (Tan et al., 2020, Lobo et al., 2022, Jubber et al., 2023). Pioglitazone (Actos) is used D.M., Actos is an oral diabetes medicine that helps control blood sugar levels. The use of Pioglitazone has been associated with an increased risk of bladder cancer in some studies, leading to regulatory warnings and recommendations for careful consideration of the drug's risks and benefits. The findings in the current study, showing a highly significant difference in Pioglitazone use in the control was 13.3% while in the patient group 65% with P-Value 0.0006, align with these concerns and suggest that further investigation is needed to fully understand the relationship between Pioglitazone and bladder cancer. Patients prescribed Pioglitazone should be monitored for signs of bladder cancer, such as blood in the urine or urinary symptoms, and should discuss the potential risks and benefits with their healthcare provider. These results agree with studies in other countries (Gangopadhyay and Singh, 2023, Xu et al., 2022, Grunberger, 2013, Yki-Järvinen, 2005). Table & figure describe the Lynch syndrome and other genetic syndromes between controls & patients (P = 0.048) with a statistically significant difference, results show the Lynch syndrome was more common among the patient population considered a risk

factor for the bladder cancer, Lynch Syndrome, also known as hereditary nonpolyposis colorectal cancer (HNPCC), is a genetic disorder that increases the risk of several types of cancer, particularly colorectal cancer, endometrial cancer, and cancers of the urinary tract, stomach, small intestine, liver, brain, and skin. It is caused by inherited mutations in mismatch repair (MMR) genes, which normally help repair DNA replication errors. The results of this study are consistent with the findings of other researchers (Dominguez-Valentin et al., 2020) this extensive study involved over 6,000 Lynch Syndrome carriers and confirmed an increased risk of various cancers, including bladder cancer. The study reported that carriers of MSH2 mutations had a higher risk of developing bladder cancer compared to the general population, which aligns with the findings of the current study.

Table (2) *(Distribution assays among studied groups.)*

Parameters			Studied groups		P – Value
			Control N = 30	Patient N = 60	
Chemicals	Yes	N	2	8	P = 0.343 NS
		%	6.7%	13.3%	
	NO	N	28	52	
		%	93.3%	86.7%	
Chronic Bladder Problems	Yes	N	10	36	P = 0.017 S
		%	33.3%	60%	
	NO	N	20	24	
		%	66.7%	40%	
Pioglitazone (Actos) use D.M	Yes	N	4	39	P = 0.0006 HS
		%	13.3%	65%	
	NO	N	26	21	
		%	86.7%	35%	
Lynch syndrome and other genetic syndromes	Yes	N	1	11	P = 0.048 S
		%	3.3%	18.3%	
	NO	N	29	49	
		%	96.7%	81.7%	

Table (3) provides a comparison of various diagnostic tests: Cytology, and ELISA (BTA, NMP-22) using several metrics: Our results show cytology and Cystoscopy both have 100% specificity, meaning they

correctly identify all true negative cases. This makes them excellent for confirming the absence of disease, while; Cytology has the lowest sensitivity at 15%, meaning it misses a significant number of true positive cases, making it less reliable as a diagnostic method, Cytology and Cystoscopy both have 100% PPV, meaning all positive test results are true positives. This is crucial for confirming the presence of bladder cancer. ELISA / BTA the sensitivity 78.3% indicates that ELISA / BTA can correctly identify 78.3% of true positive bladder cancer cases. This makes it a relatively reliable method for detecting the presence of the disease. With a specificity of 56.7%, correctly identifies 56.7% of true negative cases. This means that almost half of the negative results could be false positives, which is a limitation in terms of specificity, PPV of 78.3% means that when ELISA / BTA gives a positive result, there is a 78.3% chance that the patient has bladder cancer. This is a good predictive value, showing reliability in positive cases, the accuracy is 71.12% reflecting the overall effectiveness of BTA in correctly diagnosing both positive and negative cases. It has a reasonably high accuracy, making it a useful diagnostic tool, and an AUC of 0.792 suggests that BTA has good overall test performance, with a higher AUC indicating better diagnostic ability. On the other hand, the table shows that ELISA / NMP-22 sensitivity of 41.7% can correctly identify only 41.7% of true positive cases, which is relatively low. This limitation suggests that many cases of bladder cancer might be missed using this test alone therefore, should be used with other tests for more accuracy, A specificity of 53.3% means that the test correctly identifies 53.3% of true negative cases. This is also relatively low, indicating a higher rate of false positives, The PPV of 64.1% means that when the test is positive, there is a 64.1% chance that the patient has bladder cancer. This value is moderate, suggesting that positive results are somewhat reliable, the accuracy of 45.56% reflects the overall limited effectiveness of ELISA / NMP-22 in correctly diagnosing bladder cancer. This is relatively low. ELISA / BTA shows promise with good sensitivity and PPV, making it useful for detecting bladder cancer, especially when combined with other methods like cystoscopy. ELISA / NMP-22, however, has limitations due to low sensitivity and specificity, and its effectiveness can vary. Recent studies confirm these findings and suggest combining multiple diagnostic approaches for the best outcomes in bladder cancer detection and monitoring.

Table (3) *(Validity tests of assays by using ROC test according to clinical diagnosis (patients and controls).)*

Validity test	Cytology	ELISA / BTA	ELISA / NMP-22
Sensitivity	15%	78.3%	41.7%
Specificity	100%	56.7%	53.3%
Positive predictive value (PPV)	100%	78.3%	64.1%
Negative predictive value (NPV)	37%	56.7%	31.4%
Accuracy	43.33%	71.12%	45.56%

Area under the curve (UAC)	**0.575**	**0.792**	**0.442**
Cut-off value	**-**	**0.129**	**0.183**
P - value	**0.248 NS**	**0.0031 HS**	**0.373 NS**

CONCLUSION

Biomarkers and cytology were needed for confirmative tests such as cystoscopy and molecular diagnosis of bladder cancer.

Conflict of interests

No conflict of interest was declared by the authors.

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REFERENCES

1. SUNG, H., FERLAY, J., SIEGEL, R. L., LAVERSANNE, M., SOERJOMATARAM, I., JEMAL, A. & BRAY, F. 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 71, 209-249.
2. CHEN, W., ZHENG, R., ZENG, H., ZHANG, S. & HE, J. 2015. Annual report on status of cancer in China, 2011. Chinese journal of cancer research, 27, 2.
3. DEGERGE, K., HOLT, H. R. & HODGES, S. C. 2017. Bladder cancer: diagnosis and treatment. American Family Physician, 96, 507-514.
4. TABAYOYONG, W. & KAMAT, A. M. 2018. Current use and promise of urinary markers for urothelial cancer. Current urology reports, 19, 1-8.
5. YAFI, F. A., BRIMO, F., STEINBERG, J., APRIKIAN, A. G., TANGUAY, S. & KASSOUF, W. Prospective analysis of sensitivity and specificity of urinary cytology and other urinary biomarkers for bladder cancer. Urologic oncology: seminars and original investigations, 2015. Elsevier, 66. e25-66. e31.
6. AL-DELAIMY, W. K., AWADALLA, A., EL-ASSMY, A., ABOL-ENEIN, H. & SHOKEIR, A. 2022. Comparison of urinary telomerase, CD44, and NMP22 assays for detection of bladder squamous cell carcinoma. Current Urology, 16, 154-159.
7. MAAS, M., TODENHÖFER, T. & BLACK, P. C. 2023. Urine biomarkers in bladder cancer—Current status and future perspectives. Nature Reviews Urology, 20, 597-614.
8. JASIM MOHAMMED, B., AL-THWANI, A. N. & KANNAN, R. 2016. Demographic and genetic study for a sample of Iraqi smokers. Cancer Biology, 6, 16-27.
9. VAN HOOGSTRATEN, L. M., VRIELING, A., VAN DER HEIJDEN, A. G., KOGEVINAS, M., RICHTERS, A. & KIEMENEY, L. A. 2023. Global trends in the epidemiology of bladder cancer: challenges for public health and clinical practice. Nature reviews Clinical oncology, 20, 287-304.

10. SAGINALA, K., BARSOUK, A., ALURU, J. S., RAWLA, P., PADALA, S. A. & BARSOUK, A. 2020. Epidemiology of bladder cancer. Medical sciences, 8, 15.
11. LENIS, A. T., LEC, P. M. & CHAMIE, K. 2020. Bladder cancer: a review. Jama, 324, 1980-1991.
12. CATTO, J. W., DOWNING, A., MASON, S., WRIGHT, P., ABSOLOM, K., BOTTOMLEY, S., et al. 2021. Quality of life after bladder cancer: a cross-sectional survey of patient-reported outcomes. European urology, 79, 621-632.
13. JABER, M., ET AL. 2017. Investigation of PCR Marker Correlations in Iraqi Cancer Patients. Iraqi Journal of Medical Sciences, 14 123 - 130.
14. LOBO, N., AFFERI, L., MOSCHINI, M., MOSTAFID, H., PORTEN, S., PSUTKA, S. P., et al. 2022. Epidemiology, screening, and prevention of bladder cancer. European urology oncology, 5, 628-639.
15. TAN, W. S., TEO, C. H., CHAN, D., ANG, K. M., HEINRICH,
16. M., FEBER, A., et al. 2020. Exploring patients' experience and perception of being diagnosed with bladder cancer: a mixed-methods approach. BJU international, 125, 669-678.
17. GANGOPADHYAY, K. K. & SINGH, A. K. 2023. Will lobeglitazone rival pioglitazone? A systematic review and critical appraisal. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 17, 102747.
18. XU, B., XING, A. & LI, S. 2022. The forgotten type 2 diabetes mellitus medicine: Rosiglitazone. Diabetology international, 13, 49-65.
19. GRUNBERGER, G. 2013. Will PPAR-g agonist therapy still have a role in diabetes management in 2013? Diabetes Management, 3, 41.
20. YKI-JÄRVINEN, H. 2005. The PROactive study: some answers, many questions. The Lancet, 366, 1241-1242.
21. DOMINGUEZ-VALENTIN, M., SAMPSON, J. R., SEPPÄLÄ, T. T., TEN BROEKE, S. W., PLAZZER, J.-P., NAKKEN, S., et al. 2020. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the Prospective Lynch Syndrome Database. Genetics in Medicine, 22, 15-25.
22. SILVA-FERREIRA, M., CARVALHO, J. A., SALTA, S., HENRIQUES, T. S., RODRIGUES, P. P., MONTEIRO-REIS, S., et al. 2024. Diagnostic Test Accuracy of Urinary DNA Methylation-based Biomarkers for the Detection of Primary and Recurrent Bladder Cancer: A Systematic Review and Meta-analysis. European Urology Focu