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Original Research Article

The Investigation of NMP22 and Cytokeratin 20 Tumor Markers in Patients with Bladder Cancer

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Conflict of interest: Nil

Abstract:

Introduction and Aim: By 2040, the number of bladder tumor cases is predicted to increase to 79.6% in India. For both sexes, the lifetime risks of bladder tumors 1.1 and 0.27%, respectively. Recently, academics have become increasingly interested in marker-based detection. In bladder tumors, nuclear matrix protein (NMP) 22 and cytokeratin 20 are useful indicators that should be found and examined.

Materials and Methods: 50 male individuals (cancer patients diagnosed by TURBT and bladder biopsy) were recruited from the outpatient department of a tertiary care hospital for this cross-sectional study. The Institutional Ethics Committee granted approval for the project. ELISA was used to quantify NMP22, while immunohistochemistry (IHC) was used to measure the amounts of cytokeratin 20. While NMP 22 was estimated from 50 pre-operative bladder cancer samples, cytokeratin 20 was estimated from tissue samples of bladder cancer that had been histopathologically verified. SPSS 21.0 was used to analyze the data.

Results: Of the 50 participants, 47 were men, 45 had a medical history, and 82% had hematuria. The most prevalent type of growth on ultrasonography was polyploidal; 60% of them had sizes greater than 3 cm, and 42% showed lateral wall growth. According to histological analysis, the incidence of low- and high-grade tumors was identical, with 90% of patients showing urothelial papillary type lesions and 80% showing lamina propria invasion. While there was no discernible relationship between grade, smoking, and invasion into the lamina propria, there was a positive link between invasion into the detrusor muscle and the lymphovascular structure. Cytokeratin 20 was positive in high grade (77.8%), low grade (93.3%) tumors, diffusely positive in 24/37 and 18/19 cases of invasion into lamina propria and detrusor muscle respectively, focally positive in eight and negative in 5 cases and the association was found to be significant. Association between the immunorectivity of cytokeratin 20 , invasion into lymhovascular structures was found to be significant. The mean values of NMP 22 in high grade, with invasion into lamina propria, detrusor muscle and lympho vascular structure were found to be 17.74 U/ml, 16.15 U/ml, 18.04 U /ml and 17.75 U /ml respectively. The association of NMP 22 with high grade tumors, invasion into detrusor muscle and lympho vascular structure were found to be significant. The sensitivity, specificity of the NMP22 was 91.5% and 68.8% respectively. 31.3% of the cases were false positive and 8.8% of the cases were false negative.The connection between cytokeratin 20 and high grade (77.8%) and low grade (93.3%) cancers was shown to be significant. Cytokeratin 20 was found to be diffusely positive in 24/37 and 18/19 cases of invasion into lamina propria and detrusor muscle, respectively, and focally positive in eight and negative in five cases. There is a substantial correlation between the immunorectivity of cytokeratin 20 and its invasion of lymphovascular tissues. The mean values of NMP 22 in high grade were determined to be 17.74 U/ml, 16.15 U/ml, 18.04 U/ml, and 17.75 U/ml, respectively, with invasion into the lamina propria, detrusor muscle, and lymphovascular structure. It was discovered that NMP 22 significantly correlated with high grade malignancies, invasion of the detrusor muscle, and lymphovascular organization. The NMP22's sensitivity and specificity were 91.5% and 68.8%, respectively. False positive instances accounted for 31.3% of the total, while false negative cases made for 8.8%.

Conclusions: NMP22 and Cytokeratin 20 are potential biomarkers that could be an additional method for diagnosing bladder cancer patients and providing them with appropriate care.

Keywords: Nuclear matrix protein 22, carcinogens, cystoscopy, Keratin-20, urinary bladder neoplasms, occupation, smoking.

Introduction

Globally, the prevalence of bladder cancer is increasing, and by 2040, it is projected to represent 79.6% of cases of bladder cancer in India [1]. It is currently the ninth most common cause of death and the sixth most prevalent cancer among men. According to data from India's National Cancer Registry Programme [2], the incidence is 3.67% among men. The most typical manifestation is either microscopic or severe haematuria. At presentation, 70% of cases are classified as non-muscle invasive and 30% as muscle invasive [3]. While non-muscle invasive bladder cancer (NMIBC) has a high chance of recurrence and requires lifelong surveillance, muscle invasive bladder cancer (MIBC) is clinically aggressive and advances quickly to the stage of metastasis. Early detection and prompt screening for non-invasive breast cancer (NIMBC) aid in decisive care by lowering the chance of metastases and invasiveness. Although cystoscopy is still the gold standard for diagnosing bladder cancer, it is costly and carries a risk of UTI for the patient. In addition, there are variances in the sensitivity and specificity of CT, MRI, and cystoscopy. These modalities also have disadvantages such as invasiveness, missed diagnosis, and expensive prices. large sensitivity and specificity, cost effectiveness, ease of detection, and the ability to diagnose and detect a large proportion of cases even in pre-clinical stages are all requirements for the perfect screening or diagnostic tool.

Important structural proteins called cytokeratins create an intermediate filament network within the cytoplasm. It expresses differently in different organ tissues [4, 5]. Research indicates that the majority of benign cases have either positive or negative cytokeratin 20 (CK20) expression in the top part of the urothelium, and 36% of CIS (carcinoma in situ) patients had elevated CK20 expression [6,7]. It's also simple to collect samples from markers that are in close proximity to the uro epithelium (4). The FDA in the United States has approved detection kits for nuclear matrix protein 22 (NMP22), which is regarded as one of the significant urine biomarkers [8,9]. From DNA replication to protein translation, nuclear matrix proteins (NMPs) are an essential component of cellular nuclei and are involved in every phase of gene expression. Research indicates that bladder cancer patients have greater concentrations of NMP22 than do healthy controls. NMP22 levels have been positively correlated with tumor size, grade, and stage in certain investigations. These correlations imply that NMP22 levels may be utilized to gauge the severity of an illness and estimate prognosis [10,11]. Therefore, the goal of the current investigation was to identify NMP22 and CK 20 in male cancer patients.

Material and Methods

50 male individuals were selected from the outpatient department for this observational cross-sectional investigation, which was carried out in a tertiary care hospital in North India between December 2020 and December 2022. The study was authorized by the Institutional Ethics Committee, IEC/JNMC/434. Male participants with cancer diagnoses (TURBT and bladder biopsies) who gave their permission and were older than 14 were admitted into the study; those with a history of uroepithelial cancer, cytotoxic medication use, or radiation therapy were not. Tissue samples from bladder cancer cases with histological confirmation were used to assess cytokeratin 20.Fifty pre-operative samples from individuals with bladder cancer were collected for NMP22.Cytokeratin 20 levels were evaluated by immunohistochemistry (IHC), and NMP 22 was measured by ELISA.

Statistical Analysis

Categorical variables were presented as numbers and percentages (%) in order to do statistical analysis. Conversely, the means +/-SD were displayed for the quantitative data with a normal distribution, and the median with the 25th and 75th percentiles (interquartile range) was displayed for the data with a non-normal distribution. The Kolmogorov-Smirnov test was used to verify the normalcy of the data. In instances where the data was non-normal, non-parametric tests were employed. Excel from Microsoft and SPSS 21.0 (IBM) were used to analyze the data. Significant data was defined as P value < 0.05.

Results and Observations:

The results are depicted in the tables 1 to 5.

Table 1: Demographic profiles, cystoscopy features and histopathological findings

Parameters	Values
Age	58.94±12.60 years
Sex	Males- 47
	Females : 03
History of smoking	45 (45 males)
Occupational exposure	5
Morphology	Broad base – 27(54%)
	Polyploidal -22(44%)
Size of the tumour	>2 cms- 3(6%)
2-4cms	26 (52%)
4-6cms	18(36%)
>6cms	3(6%)
Tumour location	Anterolateral-11(22%)
	Posterolateral-17(34%)
	Anterior -1(2%)
	Posterior -5(10%)
	Lateral-14(28%)
Histopathological grade	Low garde 25 (50%)
	High grade 25 (50%)
Histopathological type	Urothelial Papillary 45 (90 %)
	Urothelial Squamous 2 (4%)
	Urothelial Nested 2 (4%)
	Inverted Papilloma 1 (2%)
Histopathological invasion in lamina propria	Diffuse 38 (76%)
	Focal 2 (4%)
	Absent 10 (20%)
Histopathological invasion in detrusor muscle	Present 20 (405)
	Absent 30 (60%)
Histopathological invasion in lymph vascular structure	Present 19 (38%)
	Absent 31 (62%)
Histological invasion of the tumor	Present 28(56 %)
	Absent 22 (41%)

Table 2; Correlations between different study variables

Correlations	r	p
Invasion and tumour morphology	0.053	0.715
Grade of tumour and history of smoking	0.064	0.067
Invasion and size of the tumour	-0.320	0.023
Grade of tumour and invasion in lamina propria	-0.470	0.001
Grade of tumour and invasion in detrusor muscle	-0.816	<0.001
Grade of tumour and its invasiveness	-0.886	<0.001

Table 3: Cross tabulation of expression of Cytokeratin 20 with the histo-pathological findings of the bladder cancer

		Expression of cytokeratin 20			P value (chi square)	Correlation
		diffuse	Focal	Negative		
Grade of the tumour	High	21	2	2	0.001	Expression of Cytokeratin with grade (r= -0.512; p<0.001)
	Low	6	14	5		
Invasion into lamina propria	present	25	8	5	0.246	Expression of Cytokeratin with invasion into lamina propria (r= 0.249; p=0.081)
	absent	2	6	2		
Invasion into detrusor muscle	Present	18	1	0	<0.001	Expression of Cytokeratin with detrusor muscle invasion (r=0.543; p<0.001)
	absent	9	14	1		
Correlation of Expression of Cytokeratin with invasion into lymph vascular structure						(r=0.511, p <0.001)
Correlation of Expression of Cytokeratin with invasiveness of the tumour						(r=0.458, p<0.001)

Table 4: Correlation of NMP 22 with study variables

	r	P value
Correlation of levels in control and study group	-0.360	0.01
With grade of tumour (mean value of NMP 22 in low and high grade tumour was found to be 12.01±2.62 and 17.74±3.39 respectively and difference was significant p<0..01)	0.682	0.001
Correlation with invasion in lamina propria **NMP 22 levels as per invasion (difference was non-significant p=0.787):** Diffuse 16.15±3.81 Focal 11.90± 0.70 Absent 10.30± 2.40	-0.578	<0.001
Correlation with invasion in detrusor muscle **Mean values of NMP 22 with invasion (p<0.001):** Present 18.04 ± 3.45 Absent 12.76 ± 3.17	-0.614	<0.001
Correlation with lympho vascular invasion **Mean values of NMP 22 with invasion (p<0.001):** Present 17.75 ± 3.75 Absent 13.10 ± 3.38	-0.540	<0.001
Correlation with invasiveness of tumour **Mean values of NMP 22 with invasion (p=0.008):** Present 17.28 ± 3.70 Absent 11.81 ± 2.33	-0.649	<0.001

Table 5: Sensitivity and specificity of NMP22 in bladder cancer

NMP 22 tested	NMP 22 positive		NMP 22 negative		Total
Positive	31	91.50%	05	31.3%	36/72%
Negative	03	8.80%	11	68.80%	14/28%
Total	34	100%	16	100%	50/100%

Discussion:

Our study's mean age of 58.94±12.60 years was consistent with the findings of Chung Un Lee et al. (2022) and Pramod Kumar et al. (2022) [13, 14]. Men are more likely to get bladder cancer than women, and this association is linked to prior occupational exposure and smoking history. Out of 50 patients, 47 were determined to be male, which is consistent with previous results [13]. Ninety percent of the patients had a history of smoking, and all of them were male. Five percent of the patients had previously worked in the paint and rubber industries. These statistics are comparable to those published by Pramod Kumar et al. in 2022. It was discovered that 44% of tumors on cystoscopy were polypoidal in nature, and 54% were broad base in nature. Additionally, we discovered that there was no significant correlation (Spearman rho p value 0.715) between the shape of the bladder tumor and its invasion into the lamina propria. In contrast to coral-like morphology, which is similar to polypoidal type in our study, crumb-like morphology—which is similar to broad base type in our study—was linked to increased invasion into deeper layers in a study by Chen et al. (2022). [15]. 66% of the cases in our analysis had tumors larger than 3 cm, with 3–4 cm, 4-5 cm, 5–6 cm, and more than 6 cm accounting for 24%, 26%, 10%, and 6% of the cases, respectively. Based on our research, there was a clinically significant association (p = 0.023) between the size of the tumor and its invasive potential. According to a study by Chen et al. (2022), 30% of cases had a tumor size greater than 3 cm, whereas 70% of cases had a tumor size less than 3 cm. The study discovered a p-value of 0.447 for the connection between invasive ness and tumor size.109 The variance in the number of patients with tumor sizes larger than three centimeters may be the cause of the disparity in significance. Sixty-six percent of the cases had a tumor larger than three centimeters, compared to thirty percent of cases in a research by Chen et al.

According to the results of our investigation, approximately 17 patients (34%) had tumors in the posterolateral wall, and 14 patients (28%) had tumors in the lateral wall. A tumor was found in the anterolateral wall in about 11 patients (22%), the posterior wall in 5 patients (10%), and the bladder neck in 2 patients (4%). 66% of the cases in our analysis had tumors larger than 3 cm, with 3–4 cm, 4-5 cm, 5–6 cm, and more than 6 cm accounting for 24%, 26%, 10%, and 6% of the cases, respectively. Based on our research, there was a clinically significant association (p = 0.023) between the size of the tumor and its invasive potential. According to a study by Chen et al. (2022), 30% of cases had a tumor size greater than 3 cm, whereas 70% of cases had a tumor size less than 3 cm. The study

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The findings of our study indicate that tumors were found in the posterolateral wall in about 17 patients (34%) and the lateral wall in about 14 patients (28%). Approximately 11 patients (22%), 5 patients (10%), and 2 patients (4%), had a tumor discovered in the posterior wall, anterolateral wall, or bladder neck. An anterior wall tumor was present in just one patient. In a study by Mohammed Ali Alga fees et al. (2022), a sample size of 3750 patients was drawn from the Saudi Cancer Registry's ten-year records up to 2018. 85.6% of the cases were determined to be multifocal, with 2.7% going to the lateral wall. The ureteric orifice had the fewest cases (0.8%) [16]. The variations in population could be the reason for the variation in the tumor's location. In our investigation, 50% of tumors were either high-grade or low-grade. According to a study by Fitra Hardian Prisnamurti et al. (2022), high grade tumors made up 63.8% of the tumors, while low grade tumors accounted for 35.6% [17]. According to our study's findings, malignancies were discovered in the lateral wall in roughly 14 patients (28%), and in the posterolateral wall in about 17 patients (34%). The posterior wall, anterolateral wall, or bladder neck were the sites of tumor discoveries in about 11 patients (22%), 5 patients (10%), and 2 patients (4%). Only one patient had a tumor on the anterior wall. The Saudi Cancer Registry's ten-year records up until 2018 were used to create a sample size of 3750 patients for a study by Mohammed Ali Alga fees et al. (2022). It was shown that 85.6% of the cases were multifocal, and 2.7% of them extended to the lateral wall. There were the fewest cases (0.8%) (16) at the ureteric orifice. The cause for the variance in the tumor's position could be due to demographic variability. Fifty percent of the tumors in our study were classified as low-grade or high-grade. A study by Fitra Hardian Prisnamurti et al. (2022) found that 35.6% of the tumors were low grade tumors, whereas 63.8% of the tumors were high grade [17]. We discovered that 90% of cases were accounted for by urothelial papillary neoplasms, with squamous cell neoplasms accounting for 4% of cases. Urothelial nested neoplasms made up 4% of the cases, while inverted papillomas made up 2%. Amin Abdulkarem Obkah et al. (2021) reported that squamous cell neoplasms accounted for 24.6% of all neoplasms, while urothelial neoplasms made for 71.5% [18]. Our study's results were consistent with those of prior investigations. About 76% of the participants in our study had lamina propria invasion, while 24% did not. In 2019, Jorge Rebola et al. reported that 25.2% of cases had invasion into the lamina propria [19]. Our study's findings are consistent with prior reports [15, 17].

Our study found that 56% of cases had muscle-invasive bladder cancer, whereas Fitra Hardian Prisnamurti et al. reported that 71.7% of cases had this type of cancer. In 38.6% of instances, there was lymphovascular invasion, and in 62% of cases, there was invasion into lymphovascular structures. It was discovered that the grade of the tumor was linked to increased likelihoods of lymphovascular invasion, involvement of the muscularis propria, and penetration into the lamina propria. Our investigation revealed that 22.2% of low-grade tumors and 77.8% of individuals with high-grade tumors exhibited diffusely significant cytokeratin 20 immunostaining. On the other hand, cytokeratin 20 stained focally positive in 93.3% of the low-grade tumors. According to the chi square test, there was a significant difference between the variables, with a p value of less than 0.001. It was discovered that cytokeratin 20 has an 84% sensitivity and a 71.4% specificity. Our study found that 56% of cases had muscle-invasive bladder cancer, whereas Fitra Hardian Prisnamurti et al. reported that 71.7% of cases had this type of cancer. In 38.6% of instances, there was lymphovascular invasion, and in 62% of cases, there was invasion into lymphovascular structures. It was discovered that the grade of the tumor was linked to increased likelihoods of lymphovascular invasion, involvement of the muscularis propria, and penetration into the lamina propria. Our investigation revealed that 22.2% of low-grade tumors and 77.8% of individuals with high-grade tumors exhibited diffusely significant cytokeratin 20 immunostaining. On the other hand, cytokeratin 20 stained focally positive in 93.3% of the low-grade tumors. According to the chi square test, there was a significant difference between the variables, with a p value of less than 0.001. It was discovered that cytokeratin 20 has an 84% sensitivity and a 71.4% specificity. A study conducted in 2014 by Shazia Mumtaz et al. revealed that, in 33 cases (68.8%) of high-grade tumors and 19 cases (40.4%) of low-grade tumors, there was diffuse positive expression of cytokeratin 20 [20]. This finding was nearly identical to our findings. According to David Dum et al. (2022), 69.7% of low grade non-invasive papillary urothelial carcinomas and 79.7% of high grade non-invasive urothelial carcinomas tested positive for cytokeratin 20, and 88.1% tested positive for G3 grade, which is comparable to our findings [21]. According to our investigation, 32 of the patients who tested positive for cytokeratin 20 expression also showed invasion of the lamina propria. Of the 32 patients, 24 showed diffuse positive cytokeratin 20 staining. However, a chi square test of 0.246 indicated that there was no significant difference between the two variables,

despite a high correlation between them (p = 0.081). According to research by Sangeeta Desaoi et al., roughly 18% of the cases had no CK20, 31.3% had localized CK20 positivity, and 43.7% had diffuse cytokeratin 20 positivity [22]. In 19 of the instances in our analysis that had invasion into the muscularis propria, cytokeratin 20 was diffusely expressed in 18 of the cases. A p value of less than 0.001 indicated that there was a significant difference between the two variables. Shazia Mumtaz et al. reported that 17 out of 70 patients had cytokeratin 20 expression, with 11 instances exhibiting diffuse cytokeratin expression. These results were nearly identical to ours. In contrast to the work by Shazia Mumtaz et al., which found no positive correlation (p value of 0.18), ours revealed a significant relationship between the expression of cytokeratin 20 and invasion into detrusor muscle (p value of less than o.oo1) [20]. The underreporting of the invasion into the detrusor muscle may be the reason for this discrepancy in the results, as there was no detrusor muscle in the histology sample.

Additionally, our investigation revealed a statistically significant relationship between the tumor's invasiveness and lympho-vascular invasion and the expression of cytokeratin 20. The research revealed that the mean NMP22 value in the 50-person study population was 14.87+/-4.17 U/ml, while the mean value in the 50-person control group was 4.41+/-1.86 U/ml. Our study's results were consistent with those of the previous studies [23].

The results of our investigation indicated that the NMP22 had a 91.2% sensitivity, 68.8% specificity, 31.3% false positive rate, and 8.8% false negative rate. In 2012, Hosseini et al. reported 78.8% sensitivity, 69.6% specificity, 85.3% negative predictive value, and 59.4% positive predictive value [24].The sensitivity and specificity reported by Jones and Campbell et al. in 2007 were 32-92% with a mean of 75% and 51-94% with a mean of 75%, respectively [25]. According to Adel Alam et al. (2009), the sensitivity was 95% and the specificity was 88.9% [26]. The results of the study demonstrated a substantial connection (p value less than 0.001) between the NMP22 readings and the tumor grade (rho value - 0.682). It was discovered that the mean value of NMP22 in low-grade tumors was 12.01+/-2.62 U/ml, whereas in high-grade tumors, it was 17.74+/-3.39 U/ml. Additionally, we discovered that the independent t test revealed a significant difference between the means, with a p value of less than 0.001.The results of our investigation indicated that the NMP22 had a 91.2% sensitivity, 68.8% specificity, 31.3% false positive rate, and 8.8% false negative rate. In 2012, Hosseini et al. reported 78.8% sensitivity, 69.6% specificity, 85.3% negative predictive value, and 59.4% positive predictive value [24]. The sensitivity and specificity reported by Jones and Campbell et al. in 2007 were 32-92% with a mean of 75% and 51-94% with a mean of 75%, respectively [25]. According to Adel Alam et al. (2009), the sensitivity was 95% and the specificity was 88.9% [26]. The results of the study demonstrated a substantial connection (p value less than 0.001) between the NMP22 readings and the tumor grade (rho value - 0.682). It was discovered that the mean value of NMP22 in low-grade tumors was 12.01+/-2.62 U/ml, whereas in high-grade tumors, it was 17.74+/-3.39 U/ml. Additionally, we discovered that the independent t test revealed a significant difference between the means, with a p value of less than 0.001.

Conclusion:

According to our research, NMP22 is a sensitive biomarker that correlates with the invasiveness and grade of bladder cancer. Similar to the other studies, Cytokeratin 20 has also demonstrated a correlation with the tumor's grade and the extent of bladder cancer's invasion. Based on these results, we draw the conclusion that NMP22 and Cytokeratin 20 both seem to be promising biomarkers that may be utilized in addition to or as a substitute for other tools in the diagnosis and successful treatment of bladder cancer patients.

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